Dynamic Models of Infectious Diseases

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Editors

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Volume 1: Vector-Borne Diseases

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Preface

Despite great advances in public health worldwide, insect vector-borne infectious diseases remain a leading cause of morbidity and mortality. Diseases that are transmitted by arthropods such as mosquitoes, sand flies, fleas, and ticks affect hundreds of millions of people and account for nearly three million deaths per year globally. Additionally, the impact of insect-transmitted diseases to agriculture exceeds $100 billion annually. Newly emerging patterns of certain vector-borne diseases such as malaria, West Nile encephalitis, tick-borne diseases, and dengue fever underscore the impact of arthropod-borne illnesses. Rapidly expanding patterns of global travel and commerce, coupled with evolution of pathogen resistance, have fueled deadly epidemics of vector-borne diseases in the past 5 years that have affected millions around the world.

Currently, the best methods for control of many insect-borne diseases involve the use of chemical pesticides. Such campaigns may, in the short term, yield spectacular results. Malaria was nearly eliminated from the Indian subcontinent; Chagas disease is rapidly being vanquished in some sections of Central and South America. However, insecticide campaigns are hampered in several ways. Environmental toxicity and adverse effects on human health limit the use of many chemical pesticides. Emergence of insect resistance to a wide variety of insecticides has greatly undermined their efficacy. The cost of repeated applications of pesticides is often prohibitive. Therefore, the wholesale elimination of insect pests is neither practical nor probable. Control of these scourges requires integrated efforts directed at advanced surveillance and epidemiology, vector control through novel genetic strategies, epidemic modeling, and greater understanding of human susceptibility to disease.

In almost all branches of science, research questions are answered from planned repeated experiments. But for infectious diseases, conducting experiments in communities is not ethical or possible. The retrospect epidemiological data may not help predict the future trends of the disease. Realistic mathematical models of the transmission of infectious diseases add a new dimension of information to assist in public health policy for control of the disease. These models provide a dynamic picture of disease transmission and are useful to predict the future trends of the disease. All models require realistic details and realistic parameter values. For practitioners in

this field to make a real-world difference and influence public health policy, medical experts are to be involved to ensure the realism of model structure and estimation of key parameters. Also, intelligent methods based on IT tools can help study various disease patterns.

In Volume 1 of "Dynamic Models of Infectious Diseases," we have assembled eight chapters from highly acclaimed international scientists to address several of the major insect vector-borne diseases. A diverse and interdisciplinary group of authors has been selected with expertise in clinical infectious diseases, epidemiology, molecular biology, human genetics, and mathematical modeling. Indeed, we believe this collection of chapters is unique and should provide a valuable perspective to a wide audience. Though diverse in approach, all the authors address critical elements of disease control. Myriad tools, whether in the realm of molecular engineering, genomic analysis, predictive modeling, or information technology to improve surveillance, are presented in this collection to provide the reader with a current understanding of research methods directed at control of vector-borne diseases.

Dengue, a global vector-borne disease with propensity for explosive outbreaks, is the subject of Chap. 1 by V. Sree Hari Rao and M. Naresh Kumar. This chapter focuses on evolving tools of mathematical modeling as strategies for mitigation of dengue epidemics. The authors present new predictive models aimed at better characterization of human susceptibility and disease severity.

In Chap. 2, Maia Martcheva and Olivia Prosper have presented a detailed discussion on the dynamic mathematical modeling activity of the vector-borne diseases. This work demonstrates that models involving time delays are best suited for a more realistic description of different types of dynamical behaviors associated with the transmission of these diseases.

West Nile virus, an emerging vector-borne disease, is the focus of Chap. 3 by Eleanor Deardorff and Gregory Ebel. The spread of West Nile virus by invading species of Culex mosquitoes in the USA has brought much attention to the study of vector-borne diseases, by illustrating the potential of these illnesses to impact highly industrialized regions of the world. The authors discuss the current state of the epidemic in the USA and critical aspects of vector and host biology that determines effectiveness of control measures.

Chapters 4 and 5, by Dr. Ravi Durvasula and colleagues, address leishmaniasis and Chagas disease, two vector-borne disease complexes with global impact. Current epidemiology of these diseases and the latest therapeutic approaches are outlined. Evolving paratransgenic strategies from the Durvasula Laboratory aimed at reducing competence of insect vectors to transmit pathogens are presented with a perspective of identifying novel methods for control of disease transmission.

Information technology methodologies for monitoring and control of vector-borne diseases in India provide fresh perspectives on two devastating diseases, filariasis and Japanese encephalitis, in Chaps. 6 and 7 by U. Suryanarayana Murty et al. Particular focus is given to the impact of these conditions on the Indian subcontinent and novel modeling strategies that have resulted in IT-based tools for surveillance and control of both vectors and disease transmission.

Finally, in Chap. 8, the most devastating of insect vector-borne diseases, malaria, is discussed by D.J. Perkins et al. The Perkins Laboratory is widely recognized as a leader in the study of human genetic susceptibility to deadly complications of malaria caused by *Plasmodium falciparum*. In this chapter, current research that dissects the immunological and human genetic underpinnings of malarial infection, with particular emphasis on severe malarial anemia, is reviewed with the aim of better understanding and controlling the impact of this disease in sub-Saharan Africa.

We have immense pleasure in expressing our appreciation to all those who have directly or indirectly influenced this work. Specifically, we thank all the chapter contributors and the reviewers who untiringly responded to our request by providing useful and thought-provoking reviews. We are grateful to the editorial staff at Springer, New York for their interest, initiative and enthusiasm in bringing out this publication. In particular our special thanks go to Mrs. Melanie Tucker, Editor, and Ms. Meredith Clinton, Assistant Editor, Springer Science+Business Media, New York for their very efficient handling of this manuscript.

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Chapter 1
Predictive Dynamics: Modeling for Virological Surveillance and Clinical Management of Dengue

V. Sree Hari Rao and M. Naresh Kumar

1 Introduction

Dengue fever (DF) is a mosquito-borne infectious disease caused by the viruses of the genus *Togaviridae* subgenus *Flavirus*. The disease has first appeared in the Phillipines in 1953, and from then on it has become the most important anthropod-borne viral disease due to its spread among humans (Monath 1994). The reemergence of this disease worldwide is causing larger, more frequent epidemics especially in cities and in the tropics. Dengue virus infection has been reported in more than 100 countries, with 2.5 billion people living in areas where dengue is endemic (CDC 2000; Guzman and Kouri 2002; PAHO 2007) (see Fig. 1.1). Dengue is one of the major international public health concerns of World Health Organization (WHO) because of the growing geographic distribution of virus and mosquito vectors, co-circulation of multiple virus serotypes and higher frequency of the epidemics.

The disease is caused by four distinct, but closely related viruse serotypes DEN1, DEN2, DEN3, and DEN4, which are transmitted to humans through the bites of infective female *Aedes* mosquitoes (Gubler 1998). A person who recovers from the infection due to one of the virus serotypes would have life long immunity against that serotype but he is susceptible to subsequent infection by the other three serotypes. There is strong evidence (De Paula and Fonseca 2004; Gubler 1998; Halstead 2007; Harris et al. 2000; Monath 1994; Nimmannitya 1997; Ooi et al. 2007; Wilder-Smith and Schwartz 2005) that subsequent infections would increase the risk of more acute

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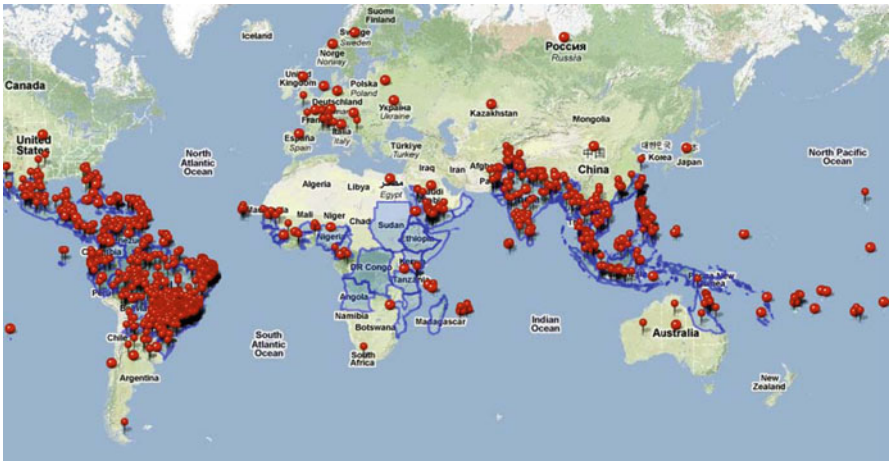

Fig. 1.1 Worldwide spread of dengue from 2007 to 2010 (CDC 2011)

forms of the disease known as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) which could be fatal and may even lead to death. The annual occurrence is estimated to be around 100 million cases of DF and 250,000 cases of DHF. The mortality rate is around 25,000 per year (Gibbons 2002). The mortality rate is most common in children. The main pathophysiology of DHF and DSS is the development of plasma leakage from the capillaries, resulting in hemoconcentration, ascites, and pleural effusion that may lead to shock (Halstead 1998).

The clinical symptoms of dengue illness overlap with other illnesses (George and Lum 1997; Harris et al. 2000; Wilder-Smith and Schwartz 2005) causing a confounding problem in disease surveillance and management (Ooi et al. 2007). Definitive laboratory diagnosis requires isolation of the virus ribonucleic acid (RNA) by polymerase chain reaction (PCR) test, immunofluorescence, or immunohistochemistry (De Paula and Fonseca 2004; Halstead 1998; Vaughn et al. 2000). Further, the places where dengue is endemic may not have the necessary infrastructure to carry out these tests (Ooi et al. 2007). Thus, a scheme for a reliable clinical diagnosis based on the data would be useful for early recognition of dengue fever.

WHO (2009) has evolved a scheme for classifying dengue infection based on the symptoms of the disease (see Table 1.1). Halstead (Halstead 2007) reviewed the clinical diagnosis and pathophysiology of vascular permeability and coagulopathy, parenteral treatment of DHF/DSS, and suggested new laboratory tests.

Recent mathematical models both deterministic (Derouich et al. 2003; Esteva and Vargas 1998, 1999; Pongsumpun and Tang 2001) and stochastic (Grassly and Fraser 2008; Medeiros et al. 2011; Paula et al. 2003; Wearing and Rohani 2006) provide an insight into the dynamics of the dengue disease. In most of the studies the incidence rates and age structure play a vital role in understanding the transmission of the virus. The rate of spread of an infectious disease which is an important aspect for disease management is estimated using a neural network technology (Sree Hari Rao and Naresh Kumar 2010). Statistical analysis based on the χ^2 tests

Table 1.1 WHO characteristics of dengue fever

Dengue fever: Headache; retro-orbital pain; myalgia; arthralgia; rashes; hemorrhagic manifestations; leukopenia and supportive dengue fever serology or occurrence at the same location and time as other confirmed cases of dengue
Dengue hemorrhagic fever. (a) fever or history of acute fever, lasting 27 days, occasionally biphasic; (b) bleeding (hemorrhagic tendencies), evidenced by at least one of the following; a positive tourniquest test (TT); petechiae, ecchymosis, or purpura; bleeding from the mucosa; gastrointestinal tract; injection sites or other locations; hemotemesis or melena; thrombocytopenia (100,000 cells/mm^3 or less). (c) Evidence of plasma leakage due to increased vascular permeability, manifested by at least one of the following: a rise in the hematocrit equal or greater than 20% above average for age, sex and population; a drop in the hemotocrit following volume-replacement treatment equal to or greater than 20% of baseline; signs of plasma leakage such as pleural effusion; ascites, and hypoproteinemia
Dengue shock syndrome: Fever; hemorrhagic tendencies; thrombocytopenia, and plasma leakage must all be present plus evidence of circulatory failure manifested as: rapid and weak pulse; narrow pulse pressure (<20 mmHg) or hypotension for age (this is defined as systolic; pressure <80 mmHg for those less than 5 years of age, or <90 mmHg for those 5 years of age and older); cold clammy skin and restlessness

for discrete attributes, logistic regression and Mann–Whitney *U* test for continuous attributes are applied on the clinical data sets for classifying issues related to the diagnosis (Chadwick et al. 2006; Kalayanarooj et al. 1997; Ramos et al. 2009). Decision tree-based algorithms such as C4.5 have been used to differentiate dengue from non-dengue illness and predict the outcome of the disease. We have examined these issues critically and have established that our methodology yields more positive predictions when compared with those obtained by using C4.5 decision tree approach (Tanner et al. 2008).

Strategies to identify individuals likely to be in the early phase of dengue infection based on clinical features alone using the evidences or rules generated from the data would be of great help to the public health officials in prioritizing and directing patient stratification for clinical investigations and management. The authors have developed a new alternating decision tree (RNIADT for short) (Sree Hari Rao and Naresh Kumar 2011c) methodology which generates more accurate decisions rules as compared to the C4.5 decision tree (Tanner et al. 2008) and logistic regression (Chadwick et al. 2006; Ramos et al. 2009) for identifying the early clinical features that predict the diagnosis of dengue. Tanner et al. (2008) have applied C4.5 decision tree algorithm on acute febrile illness affected individuals using simple clinical and hematological parameters. Further, this study also requires laboratory features such as platelet count, crossover threshold value of a real-time PCR (RT-PCR) for dengue viral ribonucleic acid (RNA) and the presence of preexisting anti-dengue immunoglobulin G (IgG) antibodies. It is known that administration of these laboratory tests require 2–12 days (Sa-Ngasang et al. 2006; Vaughn et al. 1997) and in some cases the condition of the patient may not allow such a long wait. However, the research in Tanner et al. (2008) provides more insight into the scientific understanding of the disease prevalence among the infected individuals. From the effective clinical management point of view, it is desirable to have a methodology that helps one to identify the suspected dengue individuals from simple clinical features. This helps to reduce the spread of the disease in the community.

The main emphasis in this chapter is to present methods other than those followed conventionally by clinicians. The following are the principal objectives of the present study:

(a) To define the early clinical features of suspected dengue in children and adults which helps reduce the dengue virus transmission in a community
(b) To develop a new alternating decision tree methodology for predicting the diagnosis of dengue utilizing both clinical and laboratory features and to compare with other approaches based on statistical methods, logistic regression, and decision tree algorithms such as C4.5
(c) To examine the conformability of the WHO definitions of dengue fever on the realistic clinical and laboratory data
(d) To develop an accurate model which can predict the diagnosis of dengue based on clinical and laboratory features

In order to achieve this, we have used the data sets having 1,044 data records of dengue affected populations consisting of both children and adults from central and western States of India.

2 Dengue Virus Biology

The following details concerning the dengue virus and Dengue virus biology may be found in Net DV (2011). For the sake of brevity we present the following details (Net DV (2011)).

The size of the dengue virus is around 50 nm and is enveloped with a lipid membrane (Fig. 1.2). The total genome is approximately 10.6 kb in length. A short transmembrane segment attaches the viral membrane with 180 identical copies of the envelope (E) protein. The genome of the virus has about 11,000 bases that encode a single large polyprotein that is subsequently cleaved into several structural and nonstructural mature peptides. The polyprotein is divided into three structural proteins, *C*, *prM*, *E*; seven nonstructural proteins, *NS*1, *NS*2*a*, *NS*2*b*, *NS*3, *NS*4*a*, *NS*4*b*, *NS*5; and short noncoding regions on both the 5′ and 3′ ends (Fig. 1.3). The structural proteins are the capsid (C) protein, the envelope (E) glycoprotein and the membrane (M) protein, derived by furine-mediated cleavage from a prM precursor. The *E* glycoprotein is responsible for virion attachment to receptor and fusion of the virus envelope with the target cell membrane and bears the virus neutralization epitopes. In addition to the *E* glycoprotein, only one other viral protein, *NS*1, has been associated with a role in protective immunity. *NS*3 is a protease and a helicase, whereas *NS*5 is the RNA polymerase in charge of viral RNA replication.

2.1 Life Cycle of Dengue Virus

The life cycle of dengue virus involves endocytosis via a cell surface receptor (Fig. 1.4). The virus uncoats intracellularly via a specific process. In the infectious form of the virus, the envelope protein lays flat on the surface of the virus,

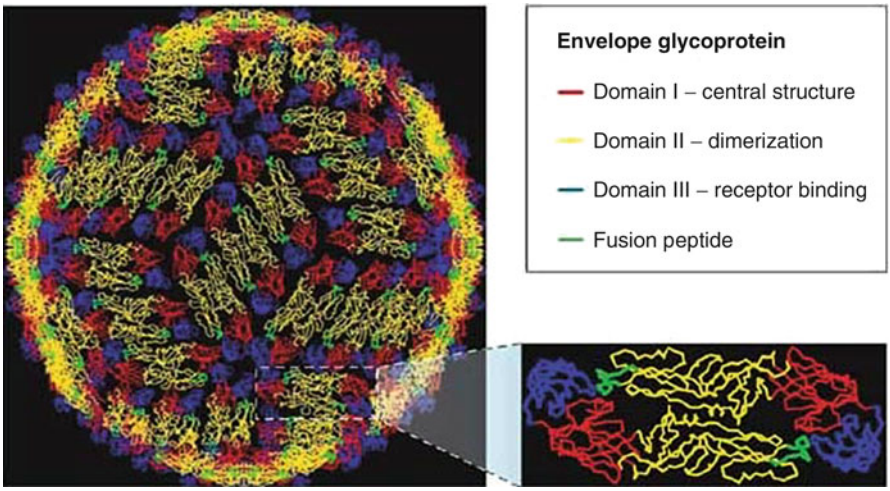

Fig. 1.2 Dengue virus particle (Stephen et al. 2007)

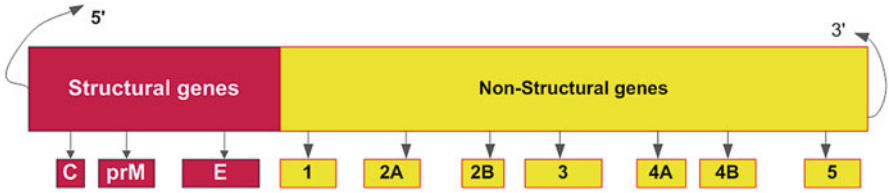

Fig. 1.3 Dengue virus genome

forming a smooth coat with icosahedral symmetry. However, when the virus is carried into the cell and into lysozomes, an acidic environment causes the protein to snap into a different shape, assembling into trimeric spike. Several hydrophobic amino acids at the tip of this spike inserts into the lysozomal membrane and causes the virus membrane to fuse with lysozome. This releases the RNA into the cell and infection starts.

The dengue virus (DENV) RNA genome in the infected cell is translated by the host ribosomes. The resulting polyprotein is subsequently cleaved by cellular and viral proteases at specific recognition sites. The viral nonstructural proteins use a negative-sense intermediate to replicate the positive-sense RNA genome, which then associates with the capsid protein and is packaged into individual virions. Replication of all positive-stranded RNA viruses occurs in close association with virus-induced intracellular membrane structures. DENV also induces such extensive rearrangements of intracellular membranes, called replication complex (RC). These RCs seem to contain viral proteins, viral RNA, and host cell factors. The subsequently formed immature virions are assembled by budding of newly formed nucleocapsids into the lumen of the endoplasmic reticulum (ER), thereby

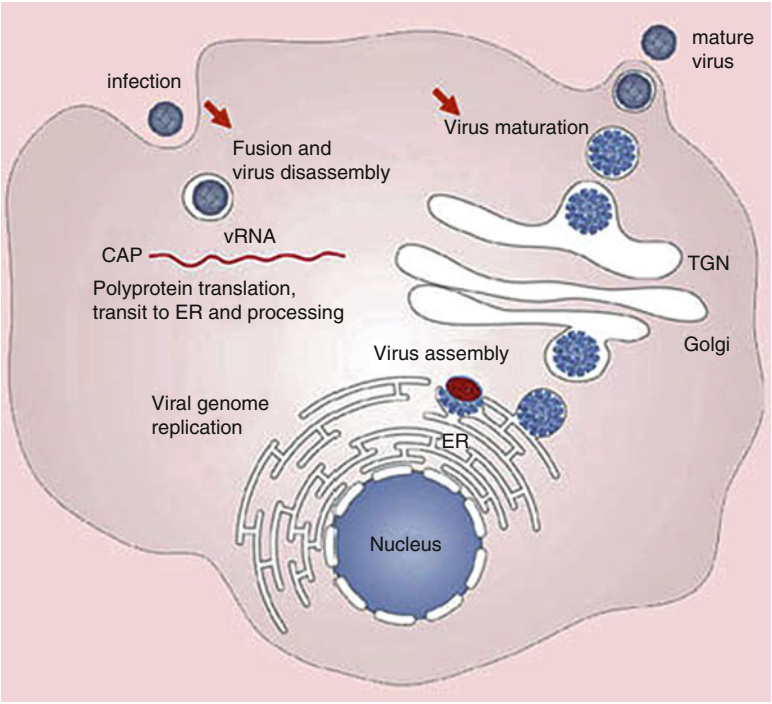

Fig. 1.4 Dengue virus life cycle (Net 2011)

acquiring a lipid bilayer envelope with the structural proteins *prM* and *E*. The virions mature during transport through the acidic trans-golgi network, where the *prM* proteins stabilize the *E* proteins to prevent conformational changes. Before release of the virions from the host cell, the maturation process is completed when *prM* is cleaved into a soluble *pr* peptides and virion-associated *M* by the cellular protease furin. Outside the cell, the virus particles encounter a neutral *pH*, which promotes dissociation of the *pr* peptides from the virus particles and generates mature, infectious virions. At this point the cycle repeats itself (Net DV, (2011).

3 Transmission of Dengue Virus

The dengue virus is transmitted mainly by the mosquitoes belonging to *Aedes* species. Among them the most prevalent species are *Aedes aegypti* and *Aedes albopictus*. In some of the regions in Pacific Islands and New Guinea *Aedes polynesiensis*, *Aedes scutellaris* and *Aedes pseudoscutallaris* transmit the disease. The *A. polynesiensis* in Society Islands and *Aedes niveus* in the Philippines are the other mosquitoes belonging to this species that transmit the virus (http://www.nathnac.org/pro/factsheets/dengue.htm). These mosquitoes prefer to breed close to

human habitation where water-filled receptacles, small pools that collect in discarded human waste are found. They are active during the daylight hours and they feed throughout the day indoors and during overcast weather.

The *A. aegypti* being a holometabolous insect undergoes a complete metamorphosis with an egg, larvae, pupae, and adult stage in its life cycle. The life cycle of *A. aegypti* can be completed within one-and-a-half to 3 weeks. The environmental conditions play a crucial role in deciding the adult lifespan which may range anywhere from 2 weeks to a month.

The bites of the infective female *Aedes* mosquitoes transmit the disease to humans. The main source of virus for the uninfected mosquitoes is the infected humans. The virus is acquired by the mosquitoes while probing and feeding on the blood of an infected person. The infected mosquito is capable of spreading the disease after 8–10 days of incubation. During the incubation period the virus replicates within the mosquito's salivary gland. Once the mosquito acquires the infection it is capable of spreading the disease to the end of its life. The mosquito's eggs, however, can survive for as long as 1 year and at temperatures as low as 10°C (50°F). The mosquitoes transmit the disease to a susceptible human during probing and blood feeding. There is no definitive theory to say whether a particular mosquito carries the dengue virus or not. The infected female mosquitoes through the transovarial process may also transmit the virus to their offsprings, but the role of this in sustained transmission of the virus to humans has not yet been defined.

Clinical symptoms in humans indicate the circulation of the virus, and this condition would prevail approximately around 2–7 days.

4 Clinical Epidemiology

The clinical symptoms such as malaise and headache, followed by sudden onset of fever, intense backache and generalized pains, mainly in the orbital and periarticular areas are manifested within 6 days of infection (http://www.histopathology-india.net/Dengue.htm). There would be a recurrence of fever for a day or two (saddleback fever) after a nonfebrile interval of 24–48 h. During this time skin rashes and lymphadenopathy appear in the infected humans. There is a greater risk to persons who are previously exposed to this virus as an enhanced uptake of the virus into the host cells by the antiviral antibodies which may lead to disseminated intravascular coagulation and death due to shock (hemorrhagic dengue).

4.1 Pathological Features

Biopsy studies of the rashes reveal that in the cases of nonfatal dengue, lymphocytic vasculitis is found in the dermis whereas in the cases of fatal DHF the gross findings are petechial hemorrhages in the skin, hemorrhagic effusions in the pleural, pericardial, and abdominal cavities. In many organs hemorrhage and congestion are seen. Histopathological examinations reveal hemorrhage, perivascular edema, and focal

necrosis but no evidence of vasculitis or endothelial lesions. It is observed that most of the morphologic abnormalities are due to disseminated intravascular coagulation and shock.

4.2 Serotypes

The dengue infection may spread due to any of the four known serotypes of the flavivirus. Based on the serotype of the virus spreading the infection, the dengue fever is termed DEN-1, DEN-2, DEN-3, and DEN-4. Even though the viral subtypes are closely related, they are antigenetically distinct. Therefore, a person already infected by one specific dengue serotype has lifelong homotypic immunity against a reinfection by the same serotype. In addition there will be a brief period of some partial heterotypic immunity but it does not provide permanent immunity or protection against the potential infection by any of the other serotypes. There is a possibility of having several serotypes circulating concurrently within an exposed population during epidemics. This is of vital importance in view of the fact that, dengue fever that produces some minor nonspecific viral symptoms, may also progress towards its more aggressive and often fatal form known as DHF.

Once a human being becomes infected by the bite of the *Aedes* mosquito, the incubation period is anywhere between 3 and 14 days (with an average lag time of 4–7 days), during which the viral replication takes place. The virus primarily targets the reticuloendothelial system, including dendritic cells, endothelial cells and hepatocytes (http://www.medicinemd.com/Med_articles/Dengue_fever_en.html). After 5–7 days of acute febrile illness, recovery is usually complete within 1–2 weeks.

4.3 Symptoms

The initial dengue infection may be asymptomatic and results in a nonspecific febrile illness, or it may produce complex manifestations of the classic dengue fever. A characteristic presentation of the symptoms includes sudden onset of fever, accompanied by severe frontal headaches, and joint (arthralgia), and muscle pains (myalgia). Some patients also experience nausea or vomiting and develop rashes on skin. The rashes would appear 3–5 days after the initial infection, and spreads from the torso to the extremities and the face.

Some patients, who have previously been infected by one of the dengue serotypes, may also develop bleeding and endothelial leakage upon infection with another dengue serotype. This syndrome is termed DHF. Subsequently, some patients with DHF may also develop shock (DSS), which is lethal and may lead to death of the infected person.

The symptoms of DHF and/or DSS are much more severe than in dengue fever, and usually occur within 3–7 days of the illness, coinciding with the time of decline or interruption of the phase of fever. The primary symptoms of DHF and DSS

consist of plasma leakage and bleeding. The plasma leakage is caused by an increased capillary permeability, often resulting in hemoconcentration, pleural effusions, and ascites. Bleeding is caused by capillary fragility and thrombocytopenia (a marked decrease of platelets) which may result in bleeding incidents into the skin (petechial skin hemorrhages), or even life-threatening bleeding into the gastrointestinal tract.

The DHF or DSS symptoms appear only in patients who are earlier infected by one or more of the dengue serotypes. Typically, the basic dengue fever lasts for about 6–7 days, with a trailing end of the fever curve after a small peak (biphasic fever pattern). The patient's thrombocytes (platelets) keep dropping until the patient's temperature has returned to normal.

It is found that dengue clinical symptoms share a commonality with those of others illnesses such as malaria, typhoid fever, leptospirosis, West Nile virus infection, measles, rubella, acute human immunodeficiency (AIDS) virus conversion disease, viral hemorrhagic fevers, rickettsial diseases, early severe acute respiratory syndrome (SARS), and any other disease that can manifest in the acute phase as an undifferentiated febrile syndrome.

4.4 Diagnosis

A confirmed diagnosis is established by culture of the virus, PCR tests, or serologic assays. The diagnosis of DHF is made on the basis of the following symptoms and signs: hemorrhagic manifestations; a platelet count of less than 100,000 per mm^3; and an objective evidence of plasma leakage, shown either by fluctuation of packed cell volume (greater than 20% during the course of the illness) or by clinical signs of plasma leakage, such as pleural effusion, ascites, or hypoproteinemia. Hemorrhagic manifestations without capillary leakage do not constitute DHF. Additional laboratory criteria for a positive diagnosis include one or more of the following:

- Demonstration of a fourfold or more increase in reciprocal IgG or immunoglobulin M (IgM) antibody titers to one or more dengue virus serotype antigens
- Isolation of the dengue virus from serum, plasma, or leukocytes
- Demonstration of dengue virus antigens or viral genomic sequences, derived from autopsy tissues

4.5 WHO Guidelines for Diagnosis of Dengue

WHO in 1975 established the following guidelines for the diagnosis of dengue fever:

- Fever
- Hemorrhages positive tourniquet test, spontaneous bruising, mucosal bleeding, vomiting blood or bloody diarrhea

- Thrombocytopenia less than 100,000 platelets/mm
- Plasma leakage evident by a hematocrit level of more than 20% higher than expected, or a drop of the hematocrit level by 20% or more, following IV fluid therapy; hypoproteinemia, pleural effusion and ascites (collection of fluids in the thoracic cavity and/or abdominal cavity)

In addition to the symptoms of dengue fever, DSS is defined as including the following:

- A rapid and weak pulse
- A narrow pulse pressure (<20 mmHg)
- Hypotension
- An altered mental status
- Cool and clammy skin

Dengue fever being a viral disease, there is no direct therapy available. The treatment is usually limited to supportive care. To maintain an adequate blood pressure and to prevent dehydration oral and intravenous fluids are provided. Platelet transfusions are indicated, if the platelet count falls below 20,000 per μl (normal level: 200,000–400,000 per μl), or if significant episodes of bleeding occur. Blood in the stool (melena) may indicate gastrointestinal bleeding and requires platelet and/or red blood cell transfusions. To manage the febrile episodes, acetaminophen containing drugs are preferred over aspirin, nonsteroidal anti-inflammatory drugs (NSAIDs) or corticosteroids. Patients with DHF or DSS require close observation, including intravenous (IV) fluids, such as Ringer's lactate solution, starch, dextran 40 or albumin 5%, all of which may be of value to the patient. Blood transfusions to replace blood loss or fresh frozen plasma for patients with a coagulopathy may be necessary in individual cases.

For more details we refer our readers to URL http://www.medicinemd.com/Med_articles/Dengue_fever_en.html

5 Knowledge Extraction Methods

Our notations and terminology are fairly consistent and may be understood by referring to WHO (2009) and other earlier works. Standard definitions are used to compute the specificity, sensitivity, predictive positive value, predictive negative value, and area under the curve (AUC).

5.1 Missing Values: Concerns

The missing values in databases may arise due to various reasons such as value being lost (erased or deleted) or not recorded, incorrect measurements, equipment errors, or possibly due to an expert not attaching any importance to a particular

procedure. The incomplete data can be identified by looking for null values in the data set. However, this is not always true, since missing values can appear in the form of outliers or even wrong data (i.e., out of boundaries) (Pearson 2005). Especially in medical databases, most data are collected as a by-product of patient care activities rather than from an organized research point of view (Cios and Mooree 2002). There are three main strategies for handling missing data situations. The first consists in eliminating incomplete observations, which has major limitations namely loss of substantial information, if many of the attributes have missing values in the data records (Kim and Curry 1977) and this renders introduction of biases in the data (Little and Rubin 1987). The second strategy is to treat the missing values during the data mining process of knowledge discovery and data mining (KDD) as envisaged in C4.5. The third method of handling missing values is through imputation, replacing each instance of the missing value with a probable or predicted value (Dixon 1979), which is most suitable for KDD applications, since the completed data can be used for any data mining activity.

There are numerous methods for predicting or approximating missing values. Single imputation strategies involve using the mean, median, or mode (Schafer 1997) or regression-based methods (Horton and Lipsitz 2001) to impute the missing values. Traditional approaches of handling missing values like complete case analysis, overall mean imputation and missing-indicator method (Heijden et al. 2006) can lead to biased estimates and may either reduce or exaggerate the statistical power. Each of these distortions can lead to invalid conclusions. Statistical methods of handling missing values consist of using maximum likelihood and expectation maximization algorithms (Allison 2002; Roderick and Donald 2002; Schafer 1997). Some of these methods would work only for certain types of attributes either nominal or numeric. Machine learning approaches like neural networks with genetic algorithms (Mussa and Tshilidzi 2006), neural networks with particle swarm optimization (Qiao et al. 2005) have been used to approximate the missing values. The use of neural networks comes with a greater cost in terms of computation and training. Methods like radial basis function networks, support vector machines, and principal component analysis have been utilized for estimating the missing values.

The wrapper algorithm (Sree Hari Rao and Naresh Kumar 2011c) presented in Appendix A checks for the presence of missing values, imputes them if they are present and then generates the decision tree. It follows from the above study that using a complete data set rather than an incomplete one results in better decision making in terms of identifying the right set of attributes that contribute to the diagnosis of the disease.

5.2 Statistical Procedures

The univariate statistical method such as χ^2 test is applied on the data sets to identify the patients with abnormal clinical findings with respect to the diagnosis of the disease. Logistic regression is used to develop a model for selecting the clinical

attributes that influence the diagnosis. Those clinical attributes with $p<0.2$ in the univariate statistic are included in the model with age and gender as potential confounders. The specificity, sensitivity, predictive value of both positives and negatives are computed using standard formulae to identify the clinical attributes that can distinguish dengue from other illnesses in children and adults. In addition to the above metrics a better measure known as area under the curve (AUC) score is being used in place of accuracies and error rate as it can represent the overall performance of a classifier (Huang and Ling 2005) in a robust manner. Based on the values (see Table 1.5) of the AUC one can categorize the performance of the classifier. The clinical attributes are selected either separately or in combination so as to have at least 70% positive and negative predictive values (Ramos et al. 2009). The statistical analysis is carried out using SPSS© software. The machine learning algorithms are developed using MATLAB© and Weka© softwares (Sree Hari Rao and Naresh Kumar 2011a, b, c, d).

5.3 What Are Decision Trees?

Decision trees are machine learning methods that can solve the problems of labeling or classifying data items out of a given finite set of classes using the features in the data items. Decision trees such as C4.5 (Quinlan 1993), classification and regression trees (CART), alternating decision trees (ADTree) (Freund and Mason 1999) have been used in computational biology, bioinformatics and clinical diagnosis (Middendorf 2004; Tanner et al. 2008; Wong et al. 2004). The C4.5 decision tree handles the missing values during the model induction phase of generating the tree.

Alternating decision trees are based on AdaBoost algorithm which generates rules based on the majority votes over simple weak rules (Freund and Mason 1999; Sree Hari Rao and Naresh Kumar 2011c). An alternating decision tree consists of decision nodes (splitter node) and prediction nodes which can be either an interior node or a leaf node. The tree generates a prediction node at the root and then alternates between decision nodes and further prediction nodes. Decision nodes specify a predicate condition and prediction nodes contain a single number denoting the predictive value. An instance can be classified by following all paths for which all decision nodes are true and summing the predictive value of the any prediction nodes that are traversed. A positive sum implies membership of one class and negative sum corresponds to the membership of the opposite class.

5.4 How to Generate and Interpret an Alternating Decision Tree?

To generate an alternating decision tree we apply the algorithm (see Appendix A) on the data set given in Table 1.2 specifically chosen for the purpose of demonstration. The data set has three attributes: Attribute1 $\in \{A, B, C\}$, Attribute2 $\in$ {True,

Table 1.2 An example data set for generating alternating decision tree

Attribute1	Attribute2	Decision
A	True	Class1
A	True	Class2
A	False	Class2
A	False	Class2
A	False	Class1
B	True	Class1
B	False	Class1
B	True	Class1
B	False	Class1
C	True	Class2
C	True	Class2
C	False	Class1
C	False	Class1
C	False	Class1

False}, and a decision attribute ∈ {Class1, Class2}. There are 14 instances out of which 9 belong to Class1 and 5 belong to Class2.

We designate Class1 as −1 and Class2 as +1. The initial sum of the weights with a precondition of the decision attribute being true is $W_+ = 5$ and $W_- = 9$. The initial prediction value at the root node is computed as $a = \frac{1}{2}\ln\frac{5}{9} = -0.2954$. The weights associated with these instances are then updated (see Appendix A item 3 (iv)) as $w_{i,1} = e^{0.2954} = 0.745$ for Class1 and $w_{i,1} = e^{-0.2954} = 1.341$ for Class2. We identify a weak classifier having a rule Attribute1 = *A*. There are three instances in Class2 and two instances in Class1 with Attribute1 = *A*. Therefore, the prediction value $a = \frac{1}{2}\ln\frac{(3*1.341)+1}{(2*0.745)+1} = 0.351$ and $b = \frac{1}{2}\ln\frac{(2*1.341)+1}{(7*0.745)+1} = -0.2617$. The weights are readjusted before the next boosting iteration. An alternating decision tree for the data set given in Table 1.2 is shown in Fig. 1.5. The root node indicates a predictive value of the decision tree before the splitting takes place. If the sum of all prediction values is positive then the instance belongs to the labeled Class1, otherwise it is placed in Class2. The prediction nodes are shown as ellipses and decision nodes as rectangles. The number in the ellipse indicates the boosting iteration. The dotted line connects the prediction nodes and the decision nodes, whereas a solid line connects the decision nodes with the prediction nodes. To classify an instance having attribute values Attribute1 = *A* and Attribute2 = true we first consider the root prediction value and based on the each instance value traverse the tree and add the prediction value of the particular node traversed. We derive the following sum by going down the appropriate path in the tree collecting all the prediction value encountered: −0.294 + (−0.2617) + (0.373) = −0.1827 indicating that the instance belongs to Class1.

The above methodology has been followed in Sree Hari Rao and Naresh Kumar (2011a, b, d) for identifying the early clinical features and assessment of laboratory features for dengue diagnosis and their results are presented in Sect. 6 of this chapter.

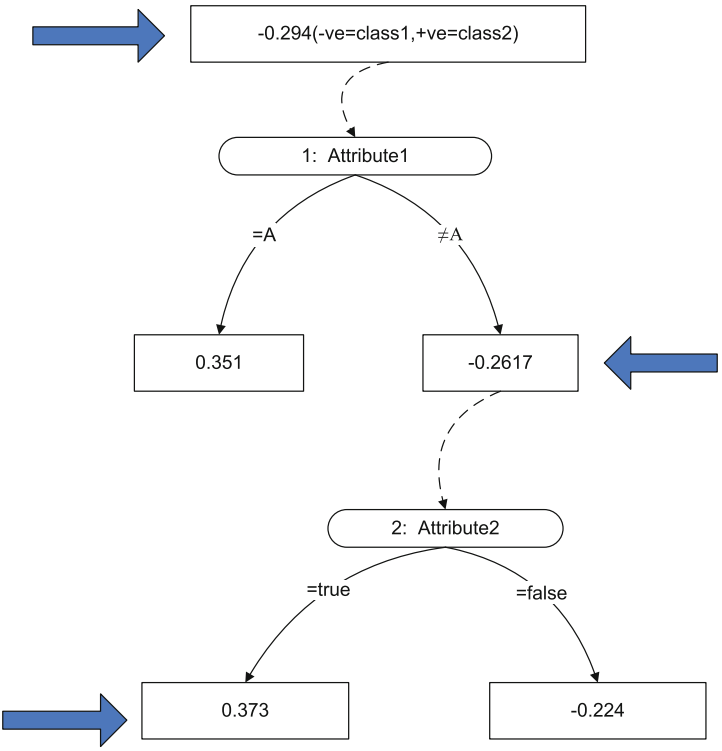

Fig. 1.5 An example alternating decision tree

5.5 What are Influential Attributes?

Decision making in databases is based on the attributes or features that form the data set. The set of attributes that contribute to better decision making are termed influential attributes. The presence of features that do not contribute much to the decision making degrades the performance accuracies of the supervised machine learning algorithms. The severity of this problem can be felt if one needs to search for patterns in large databases without considering the correlations between the attributes and the influence of such attributes on the decision attribute. The selection of influential features that maximizes the gain in the knowledge extracted from the data set is an important question in the field of machine learning, knowledge discovery, statistics and pattern recognition.

The machine learning algorithms including the top-down induction of decision trees such as classification and regression trees (CART), and C4.5 suffer from attributes that may not contribute much to decision making, thus affecting the performance of classifiers. A good choice of features would help reduce the dimensionality of the data set resulting in improved performance of the classifier in terms of accuracies and the size of the models, resulting in better understanding and interpretation.

5.6 How to Extract the Influential Attributes?

Feature selection is a popular technique to select the influential attributes as a subset of the original features. Feature selection is often used as a preprocessing step in the data mining activity. In situations presented by real world processes, influential features are often unknown a priori, hence features that are redundant or those that are weakly participating in decision making must be identified and appropriately handled.

Feature selection can be subdivided into filter-based methods and wrapper approaches. Wrapper subset evaluation models (Ron and George 1997) use the method of classification itself to measure the importance of the feature set. Wrapper methods generally result in better performance in terms of classification accuracies than filter methods because the features selected are optimized for the classification algorithm to be used. The wrapper approach (Kohavi and John 1998) defines a subset of solutions to a chosen data set and a particular induction algorithm, taking into account the inductive biases of the algorithm and its interaction with the training data set. The influential attribute selection procedure using wrapper subset evaluation is shown in Fig. 1.6. The point of concern with the wrapper method is its computational complexity as each feature set considered must be evaluated with the classification algorithm used (Dash and Liu 1997; Saeys et al. 2007).

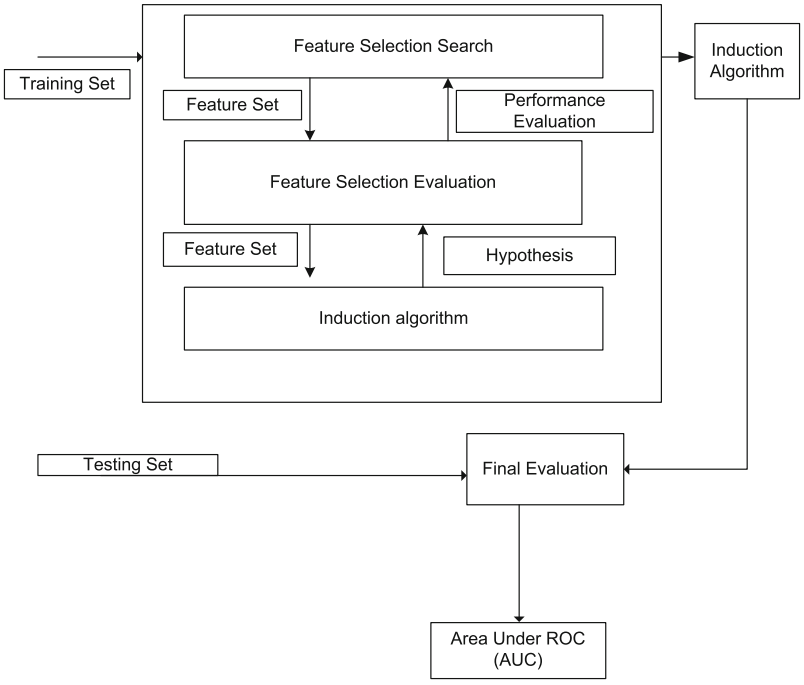

Fig. 1.6 Wrapper method of subset evaluation for selecting influencing attributes

5.7 How to Identify Optimal Feature Subsets?

5.7.1 Genetic Search

Genetic algorithms (GA) are stochastic optimization methods, inspired by the principle of natural selection. The search algorithms based on GA are capable of effectively exploring large search spaces (Goldberg 1989). GAs performs a global search as compared to many search algorithms, which perform a local or a greedy search.

A genetic algorithm is mainly composed of three operators: reproduction, crossover, and mutation. Reproduction selects good string; crossover combines good strings to try to generate better offsprings; mutation alters a string locally to attempt to create a better string. In each generation, the population is evaluated and tested for termination of the algorithm. If the termination criterion is not satisfied, the population is operated upon by the above GA operators and then reevaluated. This procedure is continued until the termination criterion is met. The default parameters for GA search (Sree Hari Rao and Naresh Kumar 2011a; Witten and Frank 2005) are given in Table 1.3. The results obtained by applying GA search (Sree Hari Rao and Naresh Kumar 2011a) for extracting influential clinical and laboratory features of dengue are discussed in Sect. 6.5 of this chapter.

5.7.2 Particle Swarm Optimization Search

The particle swarm optimization (PSO) is an evolutionary computation method which emulates the movements of flock of birds. The standard PSO consists of a randomly initialized population of size N known as particles. Each particle p_i can be viewed as a point in K dimensional space $p_i=(p_{i1}, p_{i2}, \ldots, p_{iK})$. The fitness values of the best positions of the particles at a previous time is given by $fi=(fi_1, fi_2, \ldots, fi_K)$. The index of the particle which has the best fitness value is designated as 'g_{best}'. The rate of change of position (velocity) for a particle i is represented by $V_i=(v_{i1}, v_{i2}, \ldots, v_{iK})$. The positions of the particles are updated using the following equations

$$x_{ij} = x_{ij} + v_{ij} \tag{1.1}$$

Table 1.3 Parameter values for genetic search

Attribute	Value
Start set	No attributes
Population size	20
Number of generations	20
Probability of crossover	0.6
Probability of mutation	0.033
Report frequency	20
Random number seed	1

Table 1.4 PSO search parameters

Attribute	Value
η_1	2.0
η_2	20
Max generations	50
Number of particles (N)	100

$$v_{ij} = w \times v_{ij} + \eta_1 \times \text{rand1}( ) \times (f_{ij} - x_{ij}) + \eta_2 \times \text{rand2}( ) \times (f_{gj} - x_{ij}) \tag{1.2}$$

where $j = 1, \ldots, K$, w is the inertia weight which is a positive linear function of time that changes according to the generation iteration. The parameters η_1 and η_2 represent the acceleration terms that pulls the particles towards p_{best} and g_{best}. The rand1( ) and rand2( ) are random number generation functions.

The velocities of the particles are limited by a maximum velocity $V_{\max}$. If $V_{\max}$ is too small then the particles may not explore beyond its locally good regions, i.e. they could be trapped in local optima. For the cases where $V_{\max}$ is too large the particles would fly past the good solutions.

A standard PSO search parameters are given in Table 1.4. The PSO search for extracting influential clinical and laboratory features of dengue has been utilized in Sree Hari Rao and Naresh Kumar (2011b) and their results are discussed in Sect. 6.5.

5.8 *Does Descretization of Numeric Attributes Improve Decision Making?*

Chadwick et al. (2006) have dichotomized all nominal laboratory features except WBC which was trichotomized to generate a user-friendly and accurate model.

5.8.1 Discretization Methods

Data discretization is the process of transforming quantitative attributes to qualitative attributes. Data attributes are either numeric or categorical. While categorical attributes are discrete, numerical attributes are either discrete or continuous. Discretization involves dividing an attribute values into a number of intervals $(\min_i \ldots \max_i)$ so that each interval can be treated as one value of a discrete attribute. The choice of the intervals can be determined by a domain expert or with the help of an automatic procedure.

The discretization methods such as equal width and equal frequency discretization are unsupervised and have been used because of their simplicity and reasonable effectiveness. In equal width discretization (EWD) the attribute values are divided between $x_{\min}$ and $x_{\max}$ into k equal intervals such that each cut point is

$$x_{\min} + m * \left(\frac{(x_{\max} - x_{\min})}{k}\right)$$

where m takes on the values from $0, \ldots, (k-1)$. In equal frequency discretization (EFD) each subinterval in k between x_{min} and x_{max} has approximately the same number of sorted values of the attribute. Both EWD and EFD suffer from possible attribute loss on account of the predetermined value of k.

A proportional k-interval discretization (PKI) (Yang and Webb 2001, 2002) adjusts discretization bias and variance by tuning the number and size of the interval. This strategy seeks an appropriate trade-off between the bias and variance of the probability estimation by adjusting the number and size of intervals to the number of training instances.

The authors in Sree Hari Rao and Naresh Kumar (2011a, b) have implemented the PKI algorithm on a dengue data set to convert the nominal laboratory features to categorical and evaluated the accuracies of different classifiers. The results are discussed in Sect. 6.5 of this chapter.

5.9 Standard Classification Methods

Standard machine learning classifiers such as RBFNetworks (RBF) (Haykins 1994), Bayes Network (BNT) (Friedman et al. 1997), logistic regression (LOR), Naive Bayes (NIB) (George and Pat 1995), ADTree (ADT) (Freund and Mason 1999) and C4.5 (Quinlan 1993) have been utilized in Sree Hari Rao and Naresh Kumar (2011c) to benchmark the performances of RNIADT and its efficacy in extracting knowledge from dengue data set.

5.10 Performance Metrics for Comparing Machine Classifiers

To evaluate the models generated by the decision trees, we employed a k-fold cross validation algorithm ($k=10$) as it is considered a powerful methodology to overcome data over-fitting (Kothari and Dong 2000). The data set is divided into k subsets, and the holdout method is repeated k times. Each time, one of the k subsets is used as the test set and the other $k-1$ subsets are put together to form a training set. Then the average error across all k trials is computed. To compare and evaluate the decision trees popular performance measures such as sensitivity, specificity, receiver operator characteristics (ROC), and area under ROC (AUC) (Crichton 2002; Metz 1978) have been employed. The definitions of the above measures are discussed briefly for the benefit of the readers. The classification task generates a set of rules which can be used for classifying individuals to different classes/groups. This may result in the following situations:

1. False positive (FP): the rules may predict the diagnosis of the patient as positive (presence of the disease) whereas the actual diagnosis is negative (absence of the disease).
2. False negative (FN): the rules may predict the diagnosis of the patient as negative (absence of the disease) whereas the actual diagnosis is positive (presence of the disease).

3. True positive (TP): when the prediction of the classifier matches with the actual diagnosis as positive.
4. True negative (TN): when the prediction of the classifier matches with the actual diagnosis as negative.

Based on the above situations the performance of the classifiers can be compared using the following standard measures:

(a) Sensitivity: the proportion of the people who are predicted as positive of all the people who are actually positive TP/(TP+FN).
(b) Specificity: the proportion of the people who are predicted as negative of all the people who are actually negative TN/(TN+FP).
(c) Positive predictive value: the proportion of the people whose predictions matches with the actual diagnosis as positives TP/(TP+FP).
(d) Negative predictive value: the proportion of the people whose predictions matches with the actual diagnosis as negatives TN/(TN+FN).

A theoretical, optimal prediction can achieve 100% sensitivity (i.e., predict all people from the sick group as sick) and 100% specificity (i.e., not predict anyone from the healthy group as sick).

ROC is a plot between (1−specificity) on *x*-axis and sensitivity on *y*-axis. The AUC is a measure of overall performance of the algorithm. The accuracy of the decision tree algorithms can be evaluated using the AUC measure as given in Table 1.5.

The trade-off between the sensitivity and specificity is better captured by an ROC curve, which shows how sensitivity and specificity of a model vary with some tunable parameter, is related in a direct and natural way to cost/benefit analysis (Pepe 2003; Zweig and Campbell 1993) of diagnostic decision making. ROC curves allow one to distinguish among different models, depending on what model characteristics we need, and to determine which parameter values will give us the best performance for a given application.

By measuring the area under the ROC curve (AUC) (Hanley and McNeil 1982; Liu and Wu 2003) one can obtain the accuracy of the test. The larger the area, the better the diagnostic test is. If the area is 1.0, we have an ideal test because test achieves 100% sensitivity and 100% specificity. If the area is 0.5, we have a test

Table 1.5 AUC-based classification for assessing accuracy of the test results

Range	Class
0.9 < AUC < 1.0	Excellent
0.8 < AUC < 0.9	Good
0.7 < AUC < 0.8	Worthless
0.6 < AUC < 0.7	Not good
0.5 < AUC < 0.6	Failed

which has effectively 50% sensitivity and 50% specificity. In short the area measures the ability of the test to correctly classify those with and without the disease.

$$\text{AUC} = \int_0^1 \text{ROC}\ (t)dt \tag{1.3}$$

where $t = 1 - \text{specificity}$ (false positive rate) and ROC(t) is sensitivity (true positive rate). We can establish the following classification for the test.

Generally two approaches are employed for computing AUC. A nonparametric method based on constructing trapezoids under the curve as an approximation of area and a parametric method using a maximum likelihood estimator to fit a smooth curve to the data points. Huang and Ling (2005) demonstrated that AUC is a better evaluation measure than accuracy or error rate. A nonparametric method based on Mann–Whitney U statistic (actually the p statistic from the U statistic) has been applied for evaluating the classifiers (Sree Hari Rao and Naresh Kumar 2011d).

5.11 Data Set

We first propose to identify early clinical features in both children and adults having known clinical diagnosis. This would enable one to determine the suspected dengue individuals in the community. To accomplish this task the authors (Sree Hari Rao and Naresh Kumar 2011d) have considered clinical features from a data set (see Table 1.6) consisting of 1,044 individuals belonging to central and western States of India. The patient records were segregated into children (5–15 years) and adults

Table 1.6 Clinical and laboratory features of dengue pertaining to 1,044 individuals

Attribute	Type
Vomiting/nausea	No, yes
Myalgia	Yes, no
Rashes	No, yes
Bleeding site	No, yes
Headache	Yes, no
Restlessness	No, yes
Abdominal pain	No, yes
Retro-orbital pain	No, yes
Arthralgia	No, yes
Fever	Real
Fever duration	Integer
Pulse	Integer
Hemoglobin (Hb)	Real
White blood cell(WBC) count	Real
Platelet	Real
Packed cell volume (PCV)	Real
Diagnosis	Negative, Positive

(≥16 years) as the clinical symptoms presented by them are not similar (Pongsumpun and Tang 2001; Ramos et al. 2009). The data records included the demographic attributes age, gender in addition to clinical symptoms fever, fever duration, headache, retro-orbital pain (eye pain), myalgia (body pain), arthralgia (joint pain), nausea or vomiting, rashes, bleeding sites, restlessness, and abdominal pain.

Later, we develop a method to handle the clinical and laboratory features for more accurate diagnosis and identification of operating range of numeric attributes that can aid in detecting the severity of the infection in suspected dengue individuals (Sree Hari Rao and Naresh Kumar 2011a). The laboratory features hemoglobin (Hb), white blood cell count (WBC), packed cell volume (PCV), platelets were considered for analysis.

6 A Predictive Modeling Strategy

Our predictive modeling strategy is as follows: we have considered data records containing both clinical and laboratory features and known diagnosis of 1,044 individuals. As a first step we consider all these records with clinical features only and utilizing the known diagnosis we apply our RNIADT methodology to determine the essential clinical features that would help identify the suspected dengue individuals. In the next step we use both clinical and laboratory features and the decision to build a predictive ADTree which has the capability of yielding the decision rules that confirm the diagnosis. The machine knowledge obtained by studying these 1,044 data records will be useful to diagnose other individuals (based on clinical and laboratory features) where the clinical decision is unavailable.

Of the 1,044 individuals with suspected dengue, 398 were children and 646 were adults. Out of the 398 children, 93 (23.3%) were dengue positive and 305 (76.7%) were dengue negative. Of the 646 adults, 256 (39.6%) were dengue positive and 390 (60.4%) were dengue negative.

6.1 Predictive Clinical Features in Children

It was observed in Sree Hari Rao and Naresh Kumar (2011d) that dengue-positive children (average age 11.7 years) were likely to be younger than dengue-negative children (average age 12.9 years) ($p < 0.05$). No significant difference in the proportions of male or female children between the dengue-positive and dengue-negative children was observed. The average fever duration for dengue positive was higher by 2 days when compared to dengue-negative ($p < 0.05$) children. Arthralgia was reported as the common clinical symptom among dengue-positive children (Table 1.7). Retro-orbital pain was reported 90% among dengue-positive children and 64% among dengue-negative children. Rashes were reported 78% and 83% among dengue-positive and dengue-negative children, respectively. The attributes

Table 1.7 Reported clinical features of suspected dengue-positive children

Clinical feature	Dengue positive ($n=93$), n (%)	Dengue negative ($n=305$), n (%)	Crude odds ratio (95% CI)
Classic dengue			
Myalgia	33/93 (35.48)	114/305 (37.38)	0.92 (0.57–1.50)
Rashes	73/93 (78.49)	255/305 (83.61)	0.72 (0.40–1.28)
Bleeding site	2/93 (2.15)	0/305 (0.00)	0.72 (0.40–1.28)
Headache	11/93 (11.83)	197/305 (64.59)	0.07 (0.04–0.14)
Restlessness	2/93 (2.15)	0/305 (0.00)	0.07 (0.04–0.14)
Abdominal pain	14/93 (15.05)	93/305 (30.49)	0.40 (0.22–0.75)
Retro-orbital pain	84/93 (90.32)	196/305 (64.26)	5.19 (2.51–10.73)
Arthralgia	85/93 (91.40)	56/305 (18.36)	47.24 (21.64–103.13)
Gastrointestinal			
Nausea or Vomiting	60/93 (64.52)	245/305 (80.33)	0.45 (0.27–0.74)

Table 1.8 Early clinical features selected by RNIADT for predicting dengue

Decision attribute	Accuracy (%)	Sensitivity (%)	Specificity (%)	Predictive value		AUC
				Positive (%)	Negative (%)	
Children						
Arthralgia	83.91	91.4	81.6	60.2	96.8	0.83
Arthralgia, headache	95.22	79.5	100	100	94.14	0.95
Arthralgia, headache, retro-orbital pain, myalgia	96.48	86.02	99.67	98.77	95.9	0.98
Arthralgia, headache, retro-orbital pain, myalgia, abdominal pain	97.27	89.2	99.67	98.8	96.8	0.98
Adults						
Arthralgia	82.2	83.9	81.0	74.3	88.5	0.79
Arthralgia, myalgia, rashes, headache, vomiting or nausea, abdominal pain	84.98	75.3	91.3	85.0	84.9	0.88

bleeding site and restlessness were reported least number of times among dengue-positive and negative children; however, rashes and bleeding site have odds of 0.72 times higher in dengue-positive children than in dengue-negative children.

The multivariate analysis revealed that dengue-positive children were 47 times more likely to present with arthralgia than dengue-negative children. Children with myalgia were found to be five times more likely to have dengue positive than dengue negative.

The alternating decision tree algorithm generated a model having clinical features arthralgia, headache, retro-orbital pain, and myalgia with a predictive value of 98.8% for dengue positive and 96.8% for dengue negative with an AUC of 0.98 (Table 1.8). The alternating decision tree for children between 5 and 15 is shown in Fig. 1.7.

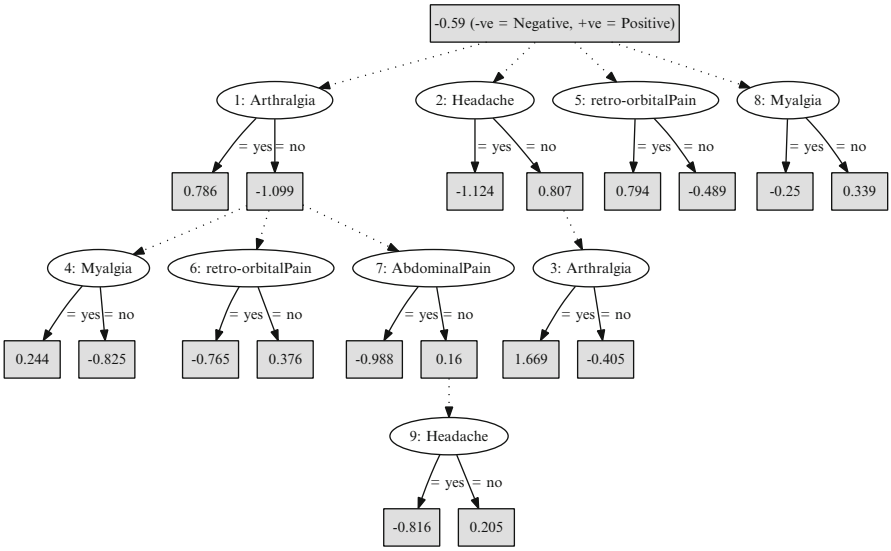

Fig. 1.7 Alternating decision tree generated based on clinical features in children

The C4.5 decision tree classifier had identified arthralgia, retro-orbital pain, headache, rashes, and abdominal pain as influential attributes with an accuracy of 90.7% and predictive positive value of 100% and negative predictive value of 89.2%. The logistic regression method when applied on the data set identified arthralgia, retro-orbital pain, bleeding site, and restlessness as having higher odds for identifying dengue positive and negative in children as compared to the other attributes. The authors have found that RNIADT has identified myalgia as an influential attribute resulting in a more accurate classifier than C4.5 and logistic regression. The authors refer the readers to Sree Hari Rao and Naresh Kumar (2011d) for a more detailed analysis and comparisons.

The decision rules extracted from an alternating decision tree for suspected dengue in children are as follows:

(a) The dominant clinical features identified are arthralgia, myalgia, retro-orbital pain.
(b) If the patient is suffering from arthralgia, retro-orbital pain, myalgia, and does not have a headache and abdominal pain then the diagnosis is positive. The predictive score is computed as $(0.786+0.807+1.669+0.244+0.794+(-0.765)+0.16+(-0.25)=3.445)$.
(c) If the patient is not suffering from arthralgia, retro-orbital pain, myalgia and if headache and abdominal pain are present then the diagnosis is negative. The score is computed as $((-1.099)+(-1.124)+(-0.405)+(-0.825)+(-0.489)+0.376+(-0.988)+0.339=-4.215)$.

6.2 Predictive Clinical Features in Adults

It has been observed that the dengue-positive adults were likely older by 3 years when compared to dengue-negative adults (average of 28.99 years vs. 25.14 years respectively) ($p < 0.05$). The proportion of patients of both the male and female population did not differ between dengue-positive and dengue-negative adults. The classic dengue symptoms most commonly reported were arthralgia, retro-orbital pain followed by myalgia and rashes (Table 1.9). Arthralgia was reported most in dengue-positive patients than in dengue-negative patients.

The multivariate analysis revealed that the dengue-positive adults were more likely to report arthralgia than dengue-negative adults. They were also likely to report myalgia than dengue-negative adults. Nausea or vomiting was found to be more likely among dengue-positive than dengue-negative adults. The odds of finding bleeding sites and retro-orbital pain are 1.8 and 1.75 times, respectively, in dengue-positive adults than in dengue-negative adults.

The RNIADT generated a model with clinical attributes arthralgia, myalgia, rashes, abdominal pain, headache, and nausea or vomiting with an accuracy of 86.2% and predictive value for positive cases as 87% and for negative is 85.7% with AUC of 0.91 (Table 1.8). The RNIADT generated for adults is shown in Fig. 1.8. The Influential attributes identified by C4.5 decision tree are arthralgia, myalgia, rashes, bleeding site, vomiting or nausea, and restlessness with an accuracy of 80.2% and predictive value of 85.2% for positives and 78.2% for dengue negatives with an AUC of 0.84. The logistic regression identified clinical features arthralgia, myalgia, retro-orbital pain, restlessness, and vomiting or nausea having higher odds with an accuracy of 77.7%, predictive value of 79.2% for positives and 77.1% for dengue negatives with an AUC of 0.78.

The following decision rules were extracted from the alternating decision tree for suspected dengue in adults:

(a) The dominant clinical features identified for positive diagnosis of dengue in adults are arthralgia and myalgia.

Table 1.9 Reported clinical features of dengue in adult patients

Clinical feature	Dengue positive ($n = 256$), n (%)	Dengue negative ($n = 390$), n (%)	Crude odds ratio (95% CI)
Classic dengue			
Myalgia	197/256 (76.95)	227/390 (58.21)	2.40 (1.68–3.41)
Rashes	213/256 (83.20)	345/390 (88.46)	0.65 (0.41–1.01)
Bleeding site	15/256 (5.86)	13/390 (3.33)	1.80 (0.84–3.86)
Headache	77/256 (30.08)	136/390 (34.87)	0.80 (0.57–1.13)
Restlessness	10/256 (3.91)	32/390 (8.21)	0.45 (0.22–0.94)
Abdominal pain	18/256 (7.03)	50/390 (12.82)	0.51 (0.29–0.90)
Retro-orbital pain	231/256 (90.23)	328/390 (84.10)	1.75 (1.07–2.86)
Arthralgia	215/256 (83.98)	74/390 (18.97)	22.39 (14.73–34.05)
Gastrointestinal			
Nausea or Vomiting	87/256 (33.98)	111/390 (28.46)	1.29 (0.92–1.82)

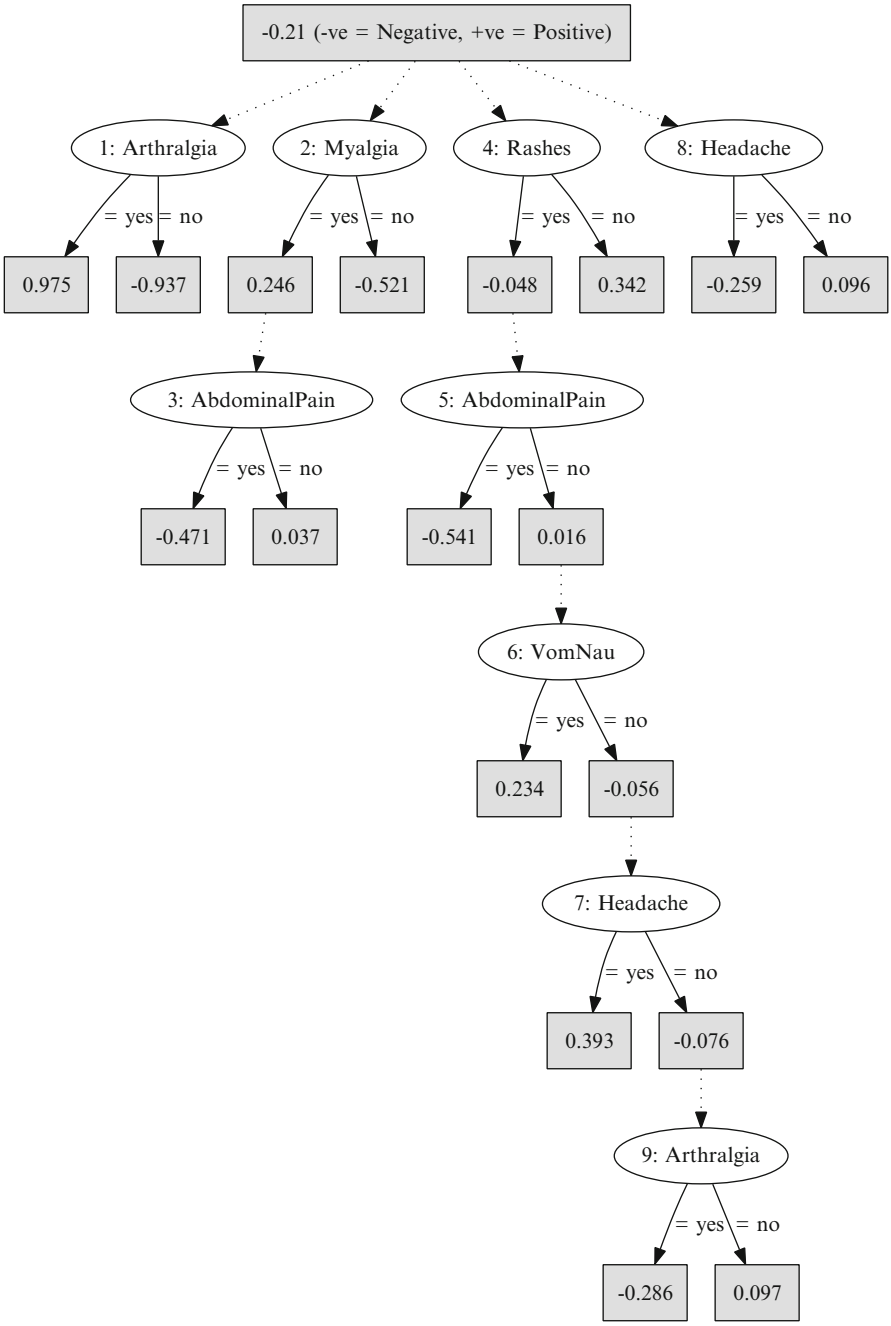

Fig. 1.8 Alternating decision tree based on clinical features in adults

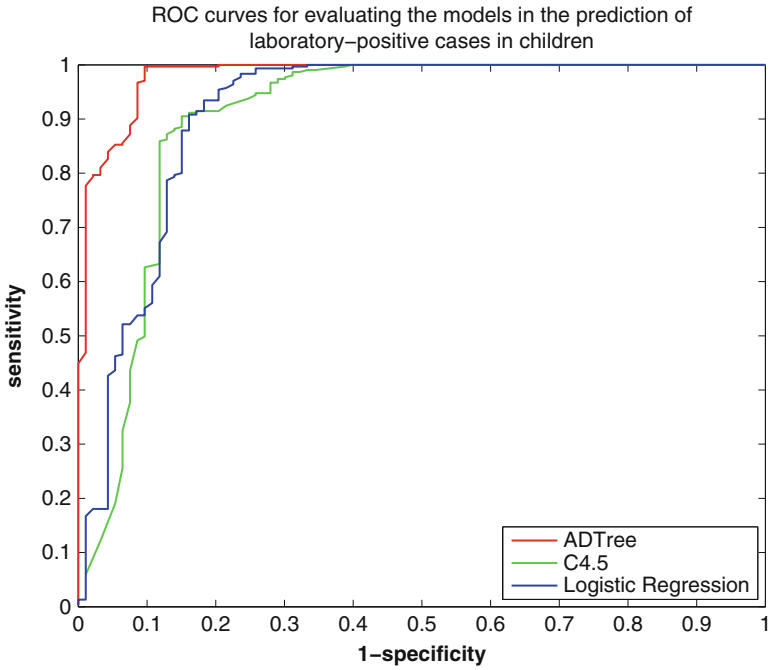

Fig. 1.9 ROC curves for evaluating the models in the prediction of dengue-positive cases in children

(b) The presence of abdominal pain is contributing for identifying negative cases of dengue.
(c) If the patient is suffering from arthralgia and myalgia and does not show signs of abdominal pain, headache, vomiting or nausea, and rashes, then he or she is dengue positive. The predictive score can be computed from the alternating decision tree as (0.975 + 0.246 + 0.037 + 0.342 + 0.016 − 0.056 −0.076 + 0.096 − 0 .286 = 1.294).
(d) If the patient is not suffering from arthralgia and myalgia but has symptoms such as abdominal pain, rashes, headache, and vomiting or nausea, then the diagnosis is negative. The predictive score is computed as (−0.937 − 0.521 − 0.4 71 − 0.048 − 0.541 + 0.234 + 0.393 − 0.259 + 0.097 = −2.053).

The receiver operator characteristic curves for RNIADT, C4.5 and logistic regression for children and adults are shown in Figs. 1.9 and 1.10, respectively. The different performance metrics suggest that RNIADT algorithm has outperformed C4.5 and logistic regression methodologies.

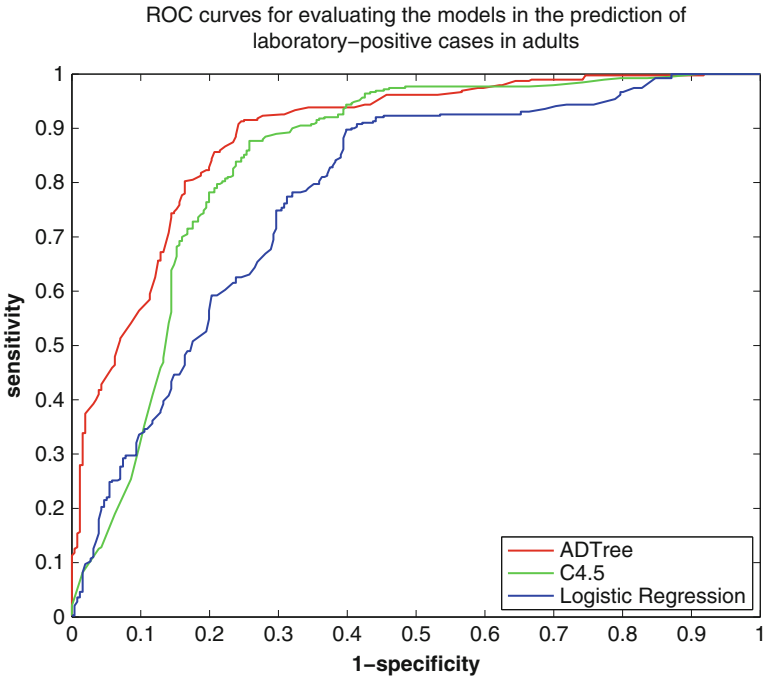

Fig. 1.10 ROC curves for evaluating the models in the prediction of dengue-positive cases in adults

6.3 Predictive Clinical and Laboratory Features in Children

The alternating decision tree identified laboratory features platelet, WBC, and Hb having 100% positive predictive value and 99.67% negative predictive value with an AUC of 0.99 (see Table 1.10). The alternating decision tree generated using the laboratory and clinical features for predicting dengue in children is shown in Fig. 1.11. Further, the laboratory attributes with platelet count less than or equal to 140, WBC over and above 8.8 and Hb less than 12.5 contributed for positive diagnosis of dengue. The clinical attributes such as fever over and above 100.5°F, pulse over and above 81.5, and the presence of arthralgia contributed for positive diagnosis.

6.4 Predictive Clinical and Laboratory Features in Adults

The alternating decision tree identified laboratory features platelet, WBC, and Hb having 100% positive predictive value and 99.24% negative predictive value with AUC of 1.0 (see Table 1.11). In adults, arthralgia (positive prediction value of 1.37)

Table 1.10 Accuracies obtained using clinical and laboratory features dengue in children

Method attribute	Accuracy (%)	Sensitivity	Specificity	Predictive value		AUC
				Positive	Negative	
RNIADT	99.75	98.92	100.00	100.00	99.67	0.99
ADT	97.74	91.40	99.67	98.84	97.44	0.99
BNT	98.74	94.62	100.00	100.00	98.39	0.99
C4.5	96.73	86.02	100.00	100.00	95.91	0.99
LOR	94.97	86.02	97.70	91.95	95.82	0.95
NIB	96.48	84.95	100.00	100.00	95.61	0.99
RBF	97.99	95.70	98.69	95.70	98.69	0.98

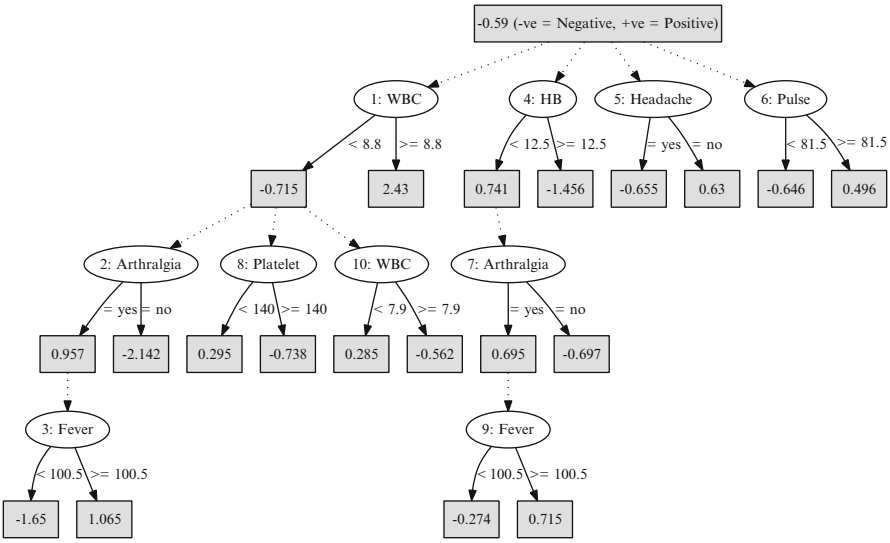

Fig. 1.11 RNIADT decision trees with predictive clinical and laboratory features of dengue in children

Table 1.11 Accuracies obtained using clinical and laboratory features dengue in adults

Method attribute	Accuracy (%)	Sensitivity	Specificity	Predictive value		AUC
				Positive	Negative	
RNIADT	99.54	98.83	100.00	100.00	99.24	1.00
ADT	97.99	96.88	98.72	98.02	97.96	1.00
BNT	95.67	89.45	99.74	99.57	93.51	0.99
C4.5	95.82	92.19	98.21	97.12	95.04	0.99
LOR	90.87	86.72	93.59	89.88	91.48	0.96
NIB	86.22	82.03	88.97	83.00	88.30	0.93
RBF	92.11	90.63	93.08	89.58	93.80	0.97

was found to be effective in diagnosis dengue. The alternating decision tree generated using the laboratory and clinical features for predicting dengue in adults is shown in Fig. 1.12. Further, the laboratory attributes with platelet less than 167.5, WBC over and above 8.9, and Hb less than 12.5 contributed for positive diagnosis

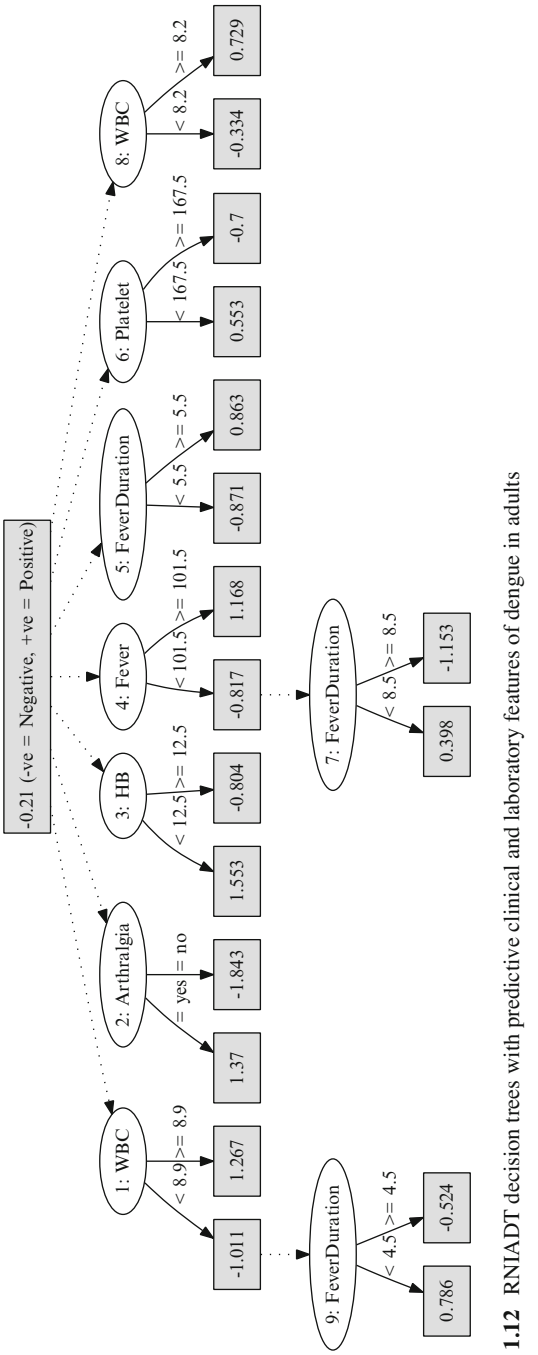

Fig. 1.12 RNIADT decision trees with predictive clinical and laboratory features of dengue in adults

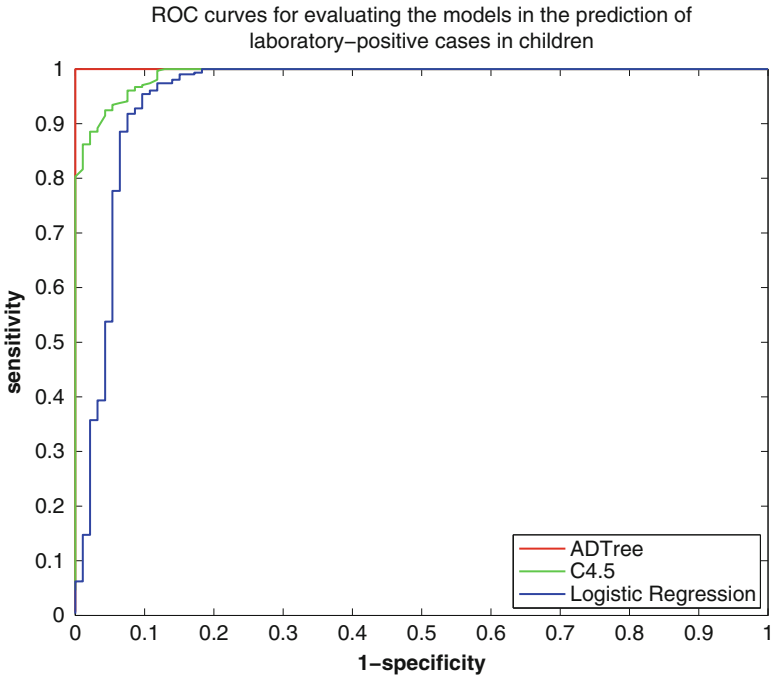

Fig. 1.13 ROC curves for evaluating the models in the prediction of dengue in children using laboratory and clinical features

of dengue. The presence of arthralgia in adults is contributed for positive predictions of dengue with a predictive value of 1.37. The clinical features such as fever over and above 101.5°F and fever duration over and above 5 days have high predictive scores for positive diagnosis of dengue.

The receiver operator characteristic curves for RNIADT, C4.5 and logistic regression for children and adults generated using clinical and laboratory features are shown in Figs. 1.13 and 1.14, respectively.

It is quite evident from ROC curves that RNIADT has outperformed C4.5 and the logistic regression methods.

6.5 Identifying Predictive Clinical and Laboratory Features Using Feature Selection Methods

A dengue data set consisting of both laboratory and clinical features has been considered in Sree Hari Rao and Naresh Kumar (2011a) (see Table 1.6) to establish more accurate and simplified decision rules. The data set had missing values up to 20% in each of the attributes. The decision tree algorithm presented in Appendix A

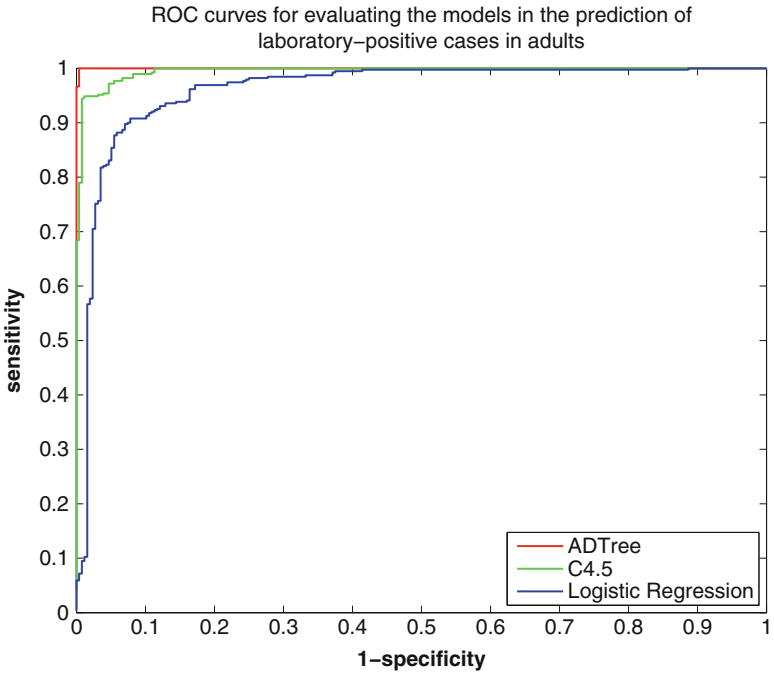

Fig. 1.14 ROC curves for evaluating the models in the prediction of dengue in adults using laboratory and clinical features

Table 1.12 Classification accuracies of different classifiers using GA search wrapper subset method

Method	RNIADT	BNT	NIB	RBF	LOR	C4.5	ADT
GA+ADT	99.71	95.40	87.07	92.34	90.23	97.03	98.75
GA+BNT	99.71	96.55	85.44	93.10	91.28	97.03	97.89
GA+NIB	99.81	93.01	86.49	91.57	90.61	96.55	97.22
GA+RBF	99.71	95.21	85.82	91.57	89.46	96.65	97.70
GA+C4.5	99.04	94.06	77.30	84.87	84.00	97.32	96.46
GA+LOR	99.90	94.16	87.07	90.13	92.05	97.03	98.37

Sree Hari Rao and Naresh Kumar (2011a, d) has been employed for generating the RNIADT and its accuracies are compared with other popular classifiers.

The authors in Sree Hari Rao and Naresh Kumar (2011a) have applied GA search algorithm for features extraction using wrapper subset evaluation procedure. These techniques were applied on dengue data set to obtain a more accurate predictive model (see Table 1.12). In Sree Hari Rao and Naresh Kumar (2011b) PSO search algorithm on dengue data set has been applied and the accuracies obtained are presented in (see Table 1.13). For a more detailed comparison of different classifiers and search algorithms the readers are referred to Sree Hari Rao and Naresh Kumar (2011a, b).

Table 1.13 Classification accuracies of different classifiers using a PSO search wrapper subset method

Method	RNIADT	BNT	NIB	RBF	LOR	C4.5	ADT
PSO+ADT	99.71	96.26	87.36	92.62	90.23	97.13	98.75
PSO+BNT	99.52	96.46	85.82	93.10	90.61	96.74	97.99
PSO+NIB	99.81	94.64	86.69	91.67	91.00	96.93	98.18
PSO+RBF	99.71	95.21	85.82	91.57	89.46	96.65	97.70
PSO+C4.5	99.71	94.16	81.23	87.16	87.26	97.32	96.74
PSO+LOR	99.71	94.25	87.36	92.15	92.24	97.03	98.37

Table 1.14 Classification accuracies of different classifiers using wrapper subset method and PKI discretization

Method	RNIADT	BNT	NIB	RBF	LOR	C4.5	ADT
PKI+GA+ADT	99.04	89.46	90.90	93.39	97.22	97.32	98.66
PKI+GA+BNT	99.90	94.06	96.17	95.79	97.32	97.70	98.66
PKI+GA+NIB	100.00	94.06	96.07	95.88	98.08	96.93	98.28
PKI+GA+RBF	99.81	93.49	94.64	95.88	96.65	97.32	98.37
PKI+GA+C4.5	99.90	88.98	91.67	90.71	97.89	97.41	95.79
PKI+GA+LOR	99.23	88.22	89.56	91.48	97.70	95.98	96.65
PKI+PSO+ADT	99.81	94.16	95.79	97.41	96.65	96.65	98.66
PKI+PSO+BNT	99.71	94.35	96.93	95.88	98.75	97.22	97.03
PKI+PSO+NIB	99.52	95.11	97.13	97.22	97.89	97.41	97.41
PKI+PSO+C4.5	100.00	92.15	93.77	95.59	97.41	97.80	97.89

Discretization method based on PKI was employed as a preprocessing step in Sree Hari Rao and Naresh Kumar (2011a) before identifying the most influential attributes. The accuracies obtained by different classifiers are shown in Table 1.14.

A comparison of the classification accuracies tabulated in Tables 1.12 and 1.13 suggests that discretization procedure improves the accuracies for the data set under consideration. It is observed in general that application of discretization method would generate user-friendly decision trees and more descriptive rules (see Fig. 1.15). The influential features identified by different methods are tabulated in Table 1.15. The RNIADT identified the attributes fever duration, pulse, WBC, and arthralgia as most influential features classified instances with a classification accuracy of 100%.

The difference in the percentage accuracy when compared with other classifiers is shown in Fig. 1.16. The RNIADT outperformed Naive Bayes, RBFNetworks, and logistic regression classifiers and the difference in accuracies were found to be greater than 7%.

The discretization method when applied on the dengue data set generated an RNIADT decision tree that outperformed Bayes Network, Naive Bayes, and RBF Network classifiers (see Fig. 1.17).

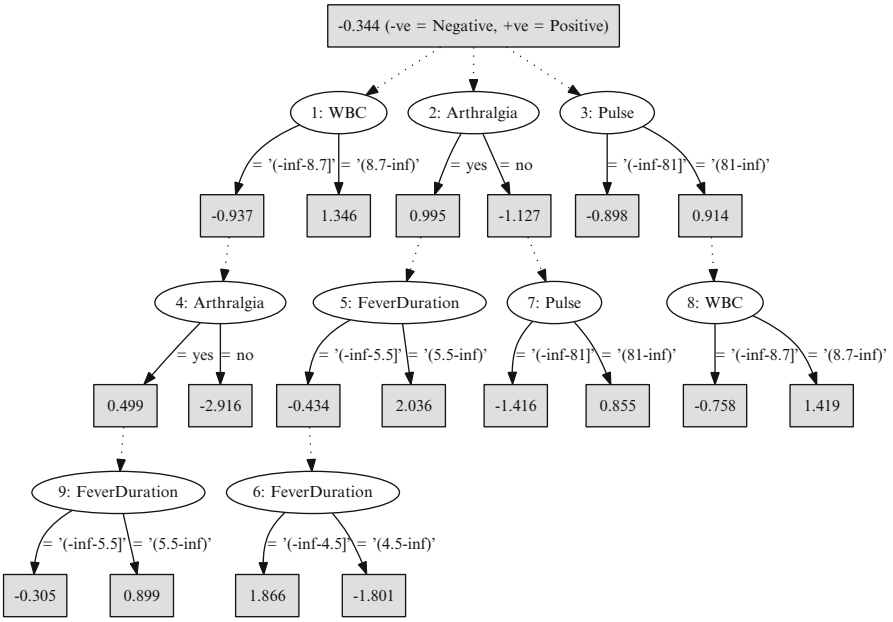

Fig. 1.15 RNIADT decision tree generated after discretization and extraction of influential attributes using a PSO search mechanism and C4.5 evaluation

Table 1.15 Influential features identified by different feature selection methods

Method	Features identified by ADT	Features identified by C4.5	Features identified by RNIADT
GA+ADT	WBC, arthralgia, Hb, fever, platelet, PCV	WBC, fever, Hb, platelets, arthralgia	Fever, platelet, arthralgia, fever duration, platelet
PKI+GA+C4.5	Hb, arthralgia, WBC, pulse, fever duration, platelet, pulse	Hb, WBC, arthralgia, platelet, pulse, fever duration, headache	WBC, arthralgia, pulse, fever duration, myalgia
PSO+C4.5	WBC, arthralgia, platelet	WBC, pulse, arthralgia, platelet, abdominalpain	WBC, arthralgia, pulse, fever duration
PKI+PSO+C4.5	WBC, arthralgia, Hb, platelet, bleeding site, pulse	WBC, Hb, arthralgia, bleeding site, platelet, pulse	WBC, arthralgia, pulse, fever duration

The ROC curves generated by different classifiers based on the dengue data set having both clinical and laboratory attributes is shown in Fig. 1.18. Figure 1.18 compares the performance of RNIADT with C4.5 and ADTree classifiers. From Fig. 1.18 we can conclude that RNIADT has outperformed the other classifiers and has a better AUC than C4.5 and ADTree.

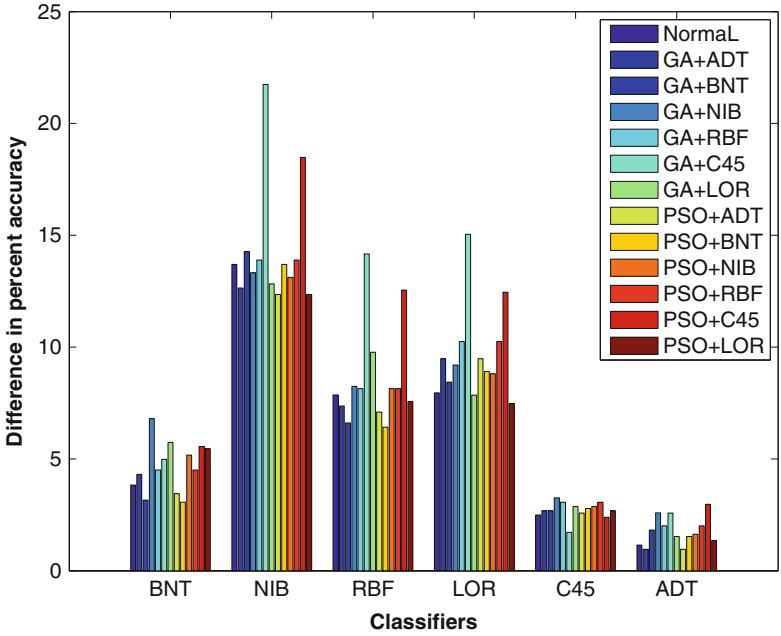

Fig. 1.16 Relative differences of other classifiers with RNIADT using different feature selection methods

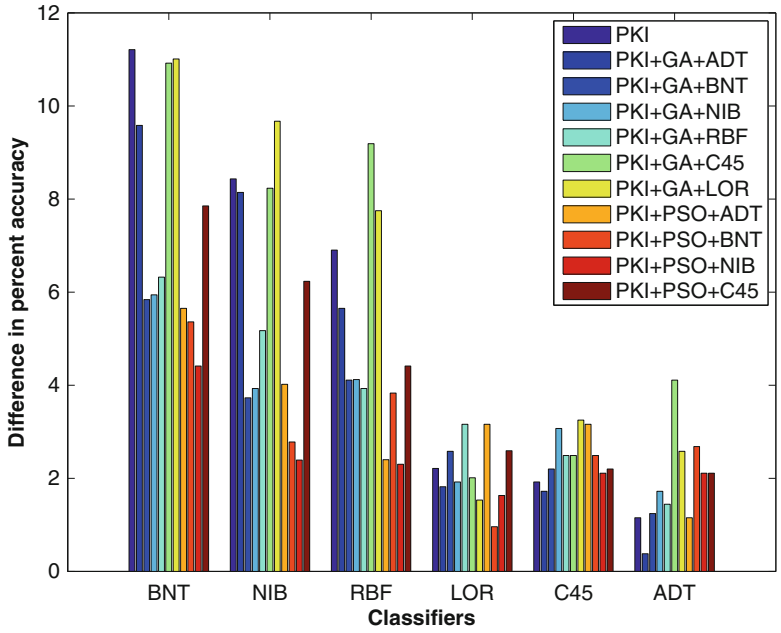

Fig. 1.17 Relative differences of other classifiers with RNIADT using different feature selection methods and PKI discretization

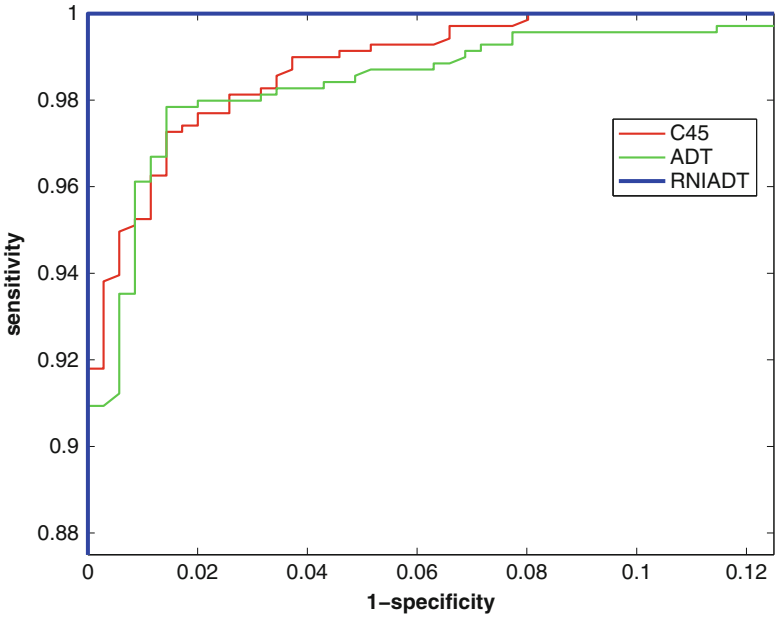

Fig. 1.18 ROC curves for classifiers trained on features extracted after discretization and using a PSO search with C4.5 evaluation procedure

7 Comparisons of Methodologies

The procedures suggested in (Chadwick et al. 2006; Ramos et al. 2009; Tanner et al. 2008) when applied on the data set (Sree Hari Rao and Naresh Kumar 2011d) (see Table 1.16) reveal the fact that the RNIADT algorithm rendered higher accuracies in terms of area under the curve and percentage predictive value for positive than those obtained by them.

Tanner et al. (2008) in their studies applied C4.5 algorithm on 1,200 patients records with data obtained in 72 h of illness. The algorithm has selected laboratory features such as platelet count, white cell count, lymphocyte, neutrophil, temperate and hematocrit as the influential attributes. The studies in Tanner et al. (2008) have suggested a WBC $\leq 6.0 \times 1{,}000$ cells with an odds ratio of 8.7 and body temperature $> 37.4°C$ mm^3 having an odds ratio of 7.2 playing a role in splitting the decision tree. Sree Hari Rao and Naresh Kumar (2011b) have identified WBC, Hb, rashes, and fever (body temperature) as the key attributes influencing the diagnosis of dengue. The predictive value of WBC $\geq 8.2 \times 1{,}000$ cells was found to be 1.3, pulse ≥ 81 has a predictive value of 0.91 mm^3 and fever duration ≥ 5.5 has a predictive value of 2.03. The comparisons of the results are presented in Tables 1.17 and 1.18. From these observations the authors have felt that the methodologies in Sree Hari Rao and Naresh Kumar (2011a, b, d) when applied on the data set (Chadwick et al. 2006; Ramos et al. 2009; Tanner et al. 2008) would yield more accurate results.

Table 1.16 Comparison of different methods for predicting early clinical features in children and adults

Method attribute	Accuracy	Sensitivity (%)	Specificity (%)	Predictive value (%)		AUC
				Positive	Negative	
Children						
Logistic regression	92.7	74.2	98.4	93.2	92.6	0.91
C4.5	90.7	60.2	100	100	89.2	0.90
RNIADT	97.2	89.3	99.7	98.8	96.8	0.98
Adults						
Logistic regression	77.7	59.9	89.7	79.2	77.1	0.78
C4.5	80.2	60.6	93.1	85.2	78.2	0.84
RNIADT	84.98	75.3	91.3	85.0	84.9	0.88

Table 1.17 Comparison of our results with (Tanner et al. 2008)

Method	True positive	False positive	False negatives	True negatives
Tanner et al. (2008)	259	83	105	753
Sree Hari Rao and Naresh Kumar (2011a)	349	0	0	695

Table 1.18 Comparison of performance measures of our methodology with Tanner et al. (2008)

Measure	Tanner et al. (2008)	Sree Hari Rao and Naresh Kumar (2011a)
Sensitivity (%)	71	100
Specificity (%)	90	100
Positive predictive value (%)	76	100
Negative predictive value (%)	88	100
AUC	0.88	0.99

8 Conclusions and Discussion

In this chapter, we have presented several methodologies that help in the effective diagnosis of the dengue illness. A first level effort leads to the question of identifying the suspected individuals in the community, which will have the major advantage of reducing transmission risk of the disease. Laboratory investigations for the confirmation of the illness on the suspected individuals will certainly help in disease management and control by providing supportive care. A new alternate decision theoretic method designated as RNIADT (which is not followed in conventional clinical treatment procedures) developed in recent times is the subject of main discussion in this chapter. This methodology has been found extremely useful in identifying the most influential clinical and laboratory characteristics of dengue illness. Further, this analysis helps one to conclude that the WHO definitions for dengue fever hold good. To substantiate, a study has been performed on a data set consisting of 1,044 individuals both children and adults where in the original definitions of

WHO are still valid. Though the methodology discussed in this chapter may be taken as a universal tool for the effective diagnosis of this disease it remains to see whether or not this methodology is geographically dependant. Though we are certain that the RNIADT methodology is universal, we could not establish the same due to lack of clinical and laboratory data pertaining to different parts of the globe. However, we are willing to share our predictive methodologies and strategies with the researchers working on dengue illness all over the globe. We hold the view that more intensive and introspective studies of this kind will pave the way for better clinical management and virological surveillance of this illness.

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9 Algorithm 1: The RNIADT Algorithm (Sree Hari Rao and Naresh Kumar 2011c)

Input: (a) Data sets for purpose of decision making $S(m, n)$ where m and n are number of records and attributes, respectively and the members of S may have missing values in any of the attributes except in the decision attribute.
(b) The type of attribute C of the columns in the data set.
(c) The number of boosting iterations T.
(d) The number of validation folds k.

Output: (a) Classification accuracy of the RNIADT for a given data set S.
(b) RNIADT consisting of a rule that is the sign of the sum of all the base rules in

$$\text{class}(x) = \text{sign}\left(\sum_{t=1}^{T} rt(x)\right)$$

Algorithm

(1) Identify and collect all records in a data set S and split them into training and testing data sets using a k fold cross validation procedure. Denote the training and testing data sets by T_k and R_k, respectively.

(2) Consider records in the training data pertaining to a particular cross fold and impute the missing values using the following procedure.

(i) Identify and collect all records in the data record set S which have missing values in one or several attributes but not those with missing values in the decision attribute. Denote this set by M i.e. $M \subseteq S$.

(ii) Pick up a record R from the set M and compute its relative distances with all members of S using the procedure given in Sree Hari Rao and Naresh Kumar (2011c). Denote this set by D.

(iii) Arrange the elements of set D in an ascending order and identify the nearest neighbors using the following procedure.

(iv) (a) Compute the score α defined as follows: $\alpha(x_k) = \frac{(x_k - median(x))}{median|x_i - median(x)|}$ where $\{x_1, x_2, \ldots, x_n\}$ denote the distances of R from R_k.

(b) Collect the data records in set S whose distances from the record R satisfies the condition $\alpha(x_k) \leq 0$. Denote this set by P.

(v) If the type of the attribute to be imputed in R is nominal or categorical, then determine the frequent item set from P using the following procedure:
  (a) Find the frequency of each categorical value of the categorical attribute.
  (b) The value to be imputed may be taken as the highest categorical value of the frequent item set obtained in Step (v) item (a).

(vi) If the type of attribute is numeric and non-integer, then determine the value to be imputed using following procedure.
  (a) Identify and collect all non-zero elements in the set D computed in Step (ii). Denote this set by B.
  (b) For each element in set B compute the quantity $\beta(j) = \frac{1}{B(j)} \forall j = 1,\ldots,\gamma$ where γ denotes the cardinality of the set B.
  (c) Compute the weight matrix as $W(j) = \frac{\beta_j}{\sum_{i=1}^{\gamma} \beta(i)} \forall j = 1,\ldots,\gamma$
  (d) The value to be imputed may be taken as $\sum_{i=1}^{j} P(j) \cdot W(j) \; \forall j = 1,\ldots,\gamma$

(vii) If the type of attribute is numeric and integer, the procedure given in Step (v) is followed.

(viii) Repeat Steps (2)(i)–(vi) for every record R in the set M.

(3) Build the ADTree on the records obtained in Step (2) as follows.

(i) Initialize the rule set R_1 to consist of the single base rule whose precondition and condition are set to True P_1 = True. The symbols P_t and R_t denote the set of preconditions and rules, respectively.

(ii) Initialize the weights of each training sample with 1 i.e.

(iii) The prediction value of the root node is calculated as $a = \frac{1}{2} \ln \frac{W_+(True)}{W_-(True)}$. $W(c)$ represents the total weight of the training samples that satisfies the base condition c. $W_+(c)$ and $W_-(c)$ denote the weights of those examples that satisfy the condition c and are labeled +1 or −1.

(iv) Pre-adjustment: re-weight the training instances using the formula $w_{i,1} = w_{i,0} \, e^{-ay_t}$ (for binary classification, the value of y_t is either +1 or −1).

(v) Perform the following steps for each boosting iteration t.
  (a) For each base condition $c_1 \in P_t$ and each condition $c_2 \in C$ calculate $Z_t(c_1, c_2) = 2(\sqrt{W_+(c_1 \wedge c_2) W_-(c_1 \wedge c_2)} + \sqrt{W_+(c_1 \wedge \sim c_2) W_-(c_1 \wedge \sim c_2)}) + W(\sim c_2)$. The set of base conditions (inequalities comparing a single feature and a constant) is denoted by C.
  (b) Select c_1, c_2 which minimizes $Z_t(c_1, c_2)$ and set R_{t+1} to be R_t with addition of rules r_t whose precondition is c_1, condition c_2 and two prediction values are $a = \frac{1}{2} \ln \frac{W_+(c_1 \wedge c_2)+1}{W_-(c_1 \wedge c_2)+1}$, $b = \frac{1}{2} \ln \frac{W_+(c_1 \wedge -c_2)+1}{W_-(c_1 \wedge -c_2)+1}$
  (c) Set P_{t+1} to be P_t with the addition of $c_1 \wedge c_2$ and $c_1 \wedge \sim c_2$
  (d) Update the weights of each training example following the equation $w_{i,t+1} = w_{i,t} \exp^{r_t(x_i) yi}$

(4) Consider the records in the testing data set pertaining to that cross fold and classify using the tree built in Step (3).

(5) Compute the percentage classification accuracy for a particular cross fold by identifying the number of correctly classified instances with the total number of instances in the testing data set.

(6) Repeat the Steps (2)–(5) for each cross fold.

(7) Compute the mean accuracy A by summing up the accuracies of each cross fold and dividing with the number of cross folds.

(8) RETURN A

(9) END

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Chapter 2
Unstable Dynamics of Vector-Borne Diseases: Modeling Through Delay-Differential Equations

Maia Martcheva and Olivia Prosper

Abstract Vector-borne diseases provide unique challenges to public health because the epidemiology is so closely tied to external environmental factors such as climate, landscape, and population migration, as well as the complicated biology of vector-transmitted pathogens. In particular, this close link between the epidemiology, the environment, and pathogen biology means that the traditional view that many vector-borne diseases are relatively stable in numerous regions does not provide a complete picture of their complexity. In fact, several regions exist with low levels of endemicity most of the time, punctuated by severe, often explosive, epidemics. These regions are considered unstable transmission settings. Ordinary differential equation (ODE) models have thus far dominated the study of vector-borne disease and have provided considerable insight into our understanding of transmission and effective control in stable transmission settings. To address the shortcomings of autonomous ODE models, we present a class of models, differential-delay equation (DDE) models, that have the potential to better describe unstable endemic settings for vector-borne disease. These models develop naturally out of the biology of diseases transmitted by vectors because of the extrinsic and intrinsic incubation periods and vector maturation process necessary for successful transmission of vector-transmitted pathogens. In this chapter, we introduce five examples of vector-borne diseases that span the globe, and discuss the clinical implications of unstable transmission of these diseases. Next, we present the original ODE version of the Ross-Macdonald model for vector-borne diseases, modify this model by introducing different types of naturally occurring delays, then illustrate how these models can exhibit more complex behavior such as oscillations via Hopf bifurcation and chaos via period-doubling, that the ODE model cannot produce. Finally, we explore

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the possibility for delay-models to contribute to our understanding of unstable transmission settings, which in turn will inform the development of effective control strategies for these epidemic-prone regions.

Keywords Vector-borne diseases • Malaria • Mathematical models • Delay-differential equations • Reproduction number • Unstable dynamics • Oscillations • Chaos

1 Introduction

During the late 19th century, it was discovered that mosquitoes are capable of transmitting diseases. Since then, arthropods have been identified as responsible for the spread of many other diseases. Although discovering this transmission mechanism led to new insights into how to better control these vector-borne diseases, more than one hundred years later, vector-borne diseases continue to pose a significant burden worldwide (Gubler 1998). The development of vector resistance to insecticides, changes in public health programs, climate change, changes in agricultural practices, the increased mobility of humans, and urban growth are all factors that contribute to the difficulty in controlling and eliminating vector-borne diseases. To further complicate matters, vector-borne diseases typically occur in developing countries with limited resources and access to health care. Because controlled epidemiological experiments are usually not possible, mathematical models have played an important role in developing a better understanding for how to mitigate the burden of these diseases. This chapter presents several examples of important vector-borne diseases, illustrating the diversity in this class of infectious diseases and consequently the need for mathematical models to address this diversity. We then discuss the difference between stable and unstable transmission settings and the implications these different settings have for public health. In Section 2.2, we introduce the Ross-Macdonald model for vector-borne diseases and consider several modifications of this model by introducing different types of delays relevant to the biology of vector-borne diseases. Finally, we discuss the contribution that these delay-differential equation models can make to better understanding unstable vector-borne disease transmission settings.

1.1 The Diversity of Vector-Borne Diseases

The formulation of mathematical models should take into consideration the epidemiology of each vector-borne disease. Some important vector-borne diseases that remain prevalent today include malaria, dengue, Chagas disease, leishmaniasis, and St. Louis encephalitis. Dengue, Chagas disease, and leishmaniasis are included in the World Health Organizations list of neglected tropical diseases (WHO). These five diseases are caused by different types of pathogens, are transmitted

by different vectors, have different clinical manifestations, result in different levels of immunity, and have different geographical distributions. To add to this complexity, while a disease may be endemic in one region, the same disease can exhibit an epidemic pattern of transmission in another region. Understanding how to model unstable transmission as well as stable transmission of vector-borne diseases is important because of the different implications that these unique transmission settings have for public health.

1.1.1 Malaria

Malaria, a disease transmitted between Anopheles mosquitoes and mammals, is considered the most important vector-borne disease (Gubler 1998), causing an estimated 190–311 million clinical episodes, and 708,000 - 1,003,000 deaths in 2008 worldwide (CDC). Malaria is responsible for the fifth greatest number of deaths due to infectious diseases and is the second leading cause of death in Africa behind HIV/AIDS (Gubler 1998). Four Plasmodium parasite species are responsible for malaria infection in humans: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae*. Of these, *P. falciparum* causes the most severe clinical symptoms and is responsible for the greatest number of deaths due to malaria infection. However, recent severe clinical cases of *P. vivax* malaria have started to change the perception that *vivax* malaria is relatively benign (Kochar et al. 2009). In fact, cases of *P. vivax* monoinfection have been reported with clinical manifestations similar to those of severe infection with *P. falciparum* malaria. These severe manifestations include cerebral malaria, anemia, respiratory distress syndrome, and acute renal failure (Kochar et al. 2009). The widespread distribution of *P. vivax*, causing roughly 100-300 million clinical cases each year, is cause for concern (Kochar et al. 2009). In regions with endemic malaria, the number of clinical cases can place a significant burden on the social and economic welfare of that population (Mendis et al. 2001), even if mortality rates are fairly low. People living in regions with moderate *P. vivax* endemicity experience 10 to 30 or more episodes of malaria throughout their childhood and working life, each episode resulting in about 5 to 15 days absent from work or school. Consequently, malaria, which typically afflicts poor, developing countries, continues the cycle of poverty by hampering the education and productivity of those at risk (Mendis et al. 2001).

1.1.2 Leishmaniasis

In contrast to malaria infection in humans, which is caused by four Plasmodium species and transmitted by mosquitoes, leishmaniasis, another dangerous vector-borne disease, is caused by over 20 leishmanial parasite species and is transmitted by roughly 30 different species of sandflies. The clinical manifestations of leishmaniases can be divided into four categories: cutaneous leishmaniasis, muco-cutaneous leishmaniasis, visceral leishmaniasis (VL) or kala-azar, and post-kala-azar dermal leishmaniasis (PKDL). Cutaneous leishmaniasis is characterized by ulcers

or nodules in the skin that eventually heal spontaneously, but slowly, causing disfiguring scars. According to the World Health Organization, there are roughly 1.5 million new cases of cutaneous leishmaniasis each year (WHO). Several months or years after an initial episode of cutaneous leishmaniasis, some patients suffer from more severe ulcers that do not spontaneously heal (Chappuis et al. 2007) and can partially or completely destroy the mucous membranes of the nose, mouth, throat cavities, and surrounding tissues (WHO). This more severe clinical manifestation is called muco-cutaneous leishmaniasis (Chappuis et al. 2007, WHO). The most dangerous manifestation of leishmaniasis is visceral leishmaniasis, which is fatal if untreated (Chappuis et al. 2007). As with malaria, visceral leishmaniasis primarily affects those in less developed countries and the burden on these countries is great, with approximately 500,000 new cases arising each year, 90% of which occur in only 5 countries: India, Bangladesh, Nepal, Sudan, and northeastern Brazil (Guerin et al. 2002). 50% of visceral leishmaniasis cases occur in India, Bangladesh, and Nepal alone (Olliaro et al. 2009). Treatments for VL exist but are expensive and impractical because treatment either requires a long hospitalization for proper administration of intravenous treatment, or because patients must self-treat with an oral drug and adhere to that treatment for four weeks (Olliaro et al. 2009). Another concern is that monotherapies increase selective pressure, leading to parasite resistance (Olliaro et al. 2009). Olliaro et al. 2009 estimated that the 2006 average household cost of an episode of VL in India is US$209 - an enormous expense considering the median household income was US$49 per month. Even when treatment is administered, treated visceral leishmaniasis cases are sometimes followed (0-6 months post-treatment in Sudan and 6 months-3 years post-treatment in India) by PKDL (Chappuis et al. 2007). PKDL is characterized by highly infectious nodular lesions on the skin. These parasite-containing lesions act as a reservoir for anthroponotic (vector-to-human) VL between epidemics (Chappuis et al. 2007). While the global distribution of visceral leishmaniasis is not as expansive as the distribution of malaria, it places second (behind malaria) for the highest mortality caused by parasitic disease, resulting in more than 50,000 deaths each year, and subsequently placing an unfortunate strain on the health and well-being of the people in a few developing countries.

1.1.3 Chagas disease

Chagas disease is another parasitic infection caused by the protozoan *Trypanosoma cruzi* (Rassi Jr et al. 2010). This vector-borne disease is transmitted by the reduviid bugs of the subfamily Triatominae to humans and over 150 species of domestic animals and wild animals (Rassi Jr et al. 2010). *T. cruzi* is an enzootic disease, which only leads to infection in humans if the vector has adapted to human dwellings. The *T. cruzi* parasites reside in the feces of infected reduviid bugs. When one of these bugs takes a blood-meal from a human, it defecates on the host, allowing infected fecal matter to enter the host through the mucosa of the eye, nose, or mouth (Prata 2000). Transmission of Chagas disease can also occur through blood transfusion and

vertically from mother to child (Prata 2000, Rassi Jr et al. 2010). Unlike malaria and leishmaniasis, roughly 10% of all Chagas cases are a result of transfusions and is the primary transmission mechanism in urban areas (Prata 2000, Rassi Jr et al. 2010). 5000 to 18,000 cases per year are congenitally transmitted, and occasionally cases are a result of the consumption of contaminated food (Prata 2000, Rassi Jr et al. 2010). Most cases of Chagas disease occur in Latin America, where *T. cruzi* is endemic. However, more recently, the immigration of people from Latin America to the US, Canada, parts of Europe and the western Pacific, has led to an increase in the number of cases in these non-endemic regions (Rassi Jr et al. 2010). Chagas disease manifests in different stages. The initial phase lasts 4 to 8 weeks and is often asymptomatic. If symptoms do occur, the onset is roughly 1 to 2 weeks after acquiring the infection vectorially. Other transmission mechanisms have different incubation periods. During this acute phase, the *T. cruzi* parasite along with the host's immunoinflammatory response can cause tissue and organ damage. 5-10% of vectorially infected patients with acute symptoms do not survive the acute phase. However, in 90% of infected individuals, the acute phase will end spontaneously, even without treatment, and approximately 30-40% of those individuals will develop a chronic form of the disease usually 10-30 years later presenting as cardiac, digestive, or cardiodigestive disease. This chronic phase, called the determinate form of chronic disease, lasts for the remainder of the patient's life and can be fatal if the patient develops Chagas heart disease. The remaining 60-70% who recover from the acute phase but never develop clinical symptoms thereafter, have the intermediate form of chronic Chagas disease. These individuals have developed the antibodies against *T. cruzi*, but show none of the ailments characteristic of the determinate form. Although progress has been made to control Chagas disease in Latin America, the various mechanisms of transmission compounded with human movement continues to place several countries, including non-endemic areas, at risk (Rassi Jr et al. 2010).

1.1.4 Dengue

Not all vector-borne diseases are caused by protozoan parasites. Dengue and dengue hemorrhagic fever (DHF), a complication of Dengue, are examples of tropical vector-borne diseases caused by four serotypes (DEN-1, DEN-2, DEN-3, DEN-4) of the dengue virus (Gubler and Clark 1995). The principal vector of dengue virus is the *Aedes aegypti* mosquito (Gubler and Clark 1995). This mosquito prefers taking blood-meals from humans and typically bites during the day. Because of the *A. aegypti* mosquito's preference for biting humans (Gubler and Clark 1995) and its ability to breed in containers holding rainwater (such as tires and cisterns) (WHO), it is considered a predominantly urban vector. In 1981, the America's experienced its first major DHF outbreak resulting from importation of a new strain of DEN-2 from Southeast Asia, and by 1995, DHF spread to 14 countries in the Americas, several of which experienced endemic DHF (Gubler and Clark 1995). The geographic distribution of dengue continues to grow, resulting in roughly 50 million dengue cases worldwide each year (WHO), spanning more than 100 countries (Gubler et al. 2002).

One of the difficulties posed by dengue is the circulation of the 4 different serotypes, which do not confer immunity to one another. Consequently, an individual may become infected up to 4 times during his/her lifetime (Gubler and Clark 1995). Furthermore, a secondary dengue infection can increase the likelihood of developing DHF, a potentially lethal complication of dengue (Gubler et al. 1998).

1.1.5 St. Louis encephalitis

St. Louis encephalitis (SLE) is another example of a vector-borne disease caused by a virus. Unlike malaria, leishmaniasis, Chagas disease, and dengue, the St. Louis encephalitis virus (SLEV) is endemic to North America (Day 2001). The first known SLE epidemic occurred in 1933 and there have been at least 41 SLE outbreaks in North America since spanning from as far south as Tampa, Florida, to as north as Toronto, Canada. Different species of *Culex* mosquito are responsible for transmission in different regions of the US and southern Canada. SLEV is an enzootic disease, requiring transmission between vertebrate hosts (usually wild birds) and mosquitoes, before it becomes prevalent enough in the mosquito population to spill-over to humans. This pre-epidemic period where the number of SLEV-infected mosquitoes increases dramatically is referred to as amplification. This amplification period might coincide with seasons when there are a lot of nestling birds that are more susceptible to infection and more vulnerable to being bitten. Some nestling birds also have higher-titer viremias and remain infectious longer because of their less-developed immune systems. Symptoms of SLE infection in humans, which are most common in people over age 59, include sustained fever above 100°F, altered consciousness, or neurologic disfunction. Most infections, however, are asymptomatic. SLE epidemics do not occur yearly, but may last for months at a time, interfering with the economy of the affected region as well as the daily lives of the people. Unfortunately, outbreaks of SLE are difficult to predict. The right combination of SLEV in the environment, climatic conditions for adequate mosquito breeding and shortening of the extrinsic incubation period, and sufficient amplification hosts such as nestling birds is necessary for spillover to the human population to occur. Some studies indicate that freezes prime south Florida for SLE epidemics (Day 2001). Another study using a hydrodynamic model to predict mosquito abundance and SLEV transmission dynamics in Florida suggests that drought can facilitate the amplification of SLEV, and consequently the spillover to humans (Shaman et al. 2002).

While the burden of epidemics in North America caused by SLEV is small relative to the burden tropical vector-borne diseases place on developing countries, the complex interactions leading to these outbreaks makes the disease very unpredictable, and consequently a system to better predict the occurrence of outbreaks is of interest (Day 2001). This disease also highlights that North America, while better equipped to handle epidemics, is not immune to the problems caused by vector-borne diseases that developing countries are all too familiar with. To a lesser degree, vector-borne diseases can burden the health-care system and hinder a state's economy as they do in the developing world, meaning that constant surveillance is still necessary even in developed countries.

1.2 Stable versus Unstable Transmission and their relative impact on Public Health

Malaria provides an ideal backdrop for understanding the differences between stable and unstable transmission settings and the implications each has for public health. In many countries, malaria transmission is stable, with perhaps some peaks and valleys in prevalence throughout the year as a result of seasonality. However, within these countries, some regions may provide less than ideal conditions for the transmission of malaria, and hence experience relatively low prevalences of the disease. These low-endemicity regions are called "unstable" if long periods of low prevalence are disrupted by epidemics (Kiszewski and Teklehaimanot 2004).

In regions with stable malaria, the likelihood of acquiring multiple infections is higher than in regions with unstable malaria. As a result, many individuals become clinically immune to malaria in stable transmission regions (Kiszewski and Teklehaimanot 2004, Giha et al. 2000). In these regions, children are the age-group at greatest risk for symptomatic malaria since they lack sufficient exposures to malaria to acquire clinical immunity. In contrast, individuals of all ages in unstable transmission settings do not have the immune response that adults acquire in stable transmission regions (Kiszewski and Teklehaimanot 2004, Giha et al. 2000). Unfortunately, this lack of acquired clinical immunity can result in violent outbreaks of malaria when conditions in the region change to favor disease transmission (Kiszewski and Teklehaimanot 2004). In fact, case fatality rates are up to 10 times greater during an epidemic in an unstable transmission region than in a stable region for the most part because the clinical manifestations of the disease are much more severe in individuals who have not developed immunity. Transmission intensity is negatively correlated with the severity of disease in children. Children are still at greatest risk in unstable regions as they are in stable regions, however when severe malaria does occur in slightly older individuals (8-15 year-olds), these patients are more likely to develop cerebral malaria. The lack of acquired immunity in epidemic-prone areas results in a more even distribution of clinical cases across age groups (Kiszewski and Teklehaimanot 2004).

Public health facilities in regions with unstable malaria are not prepared for the surge in cases during epidemics. Instead, these facilities tend to adapt to a patient load typical of an inter-epidemic period when transmission is fairly low. Once an outbreak erupts, the patient load strains the capacity of health facilities and depletes health facilities of the resources necessary to properly care for the clinically ill. The combination of low-immunity to malaria in patients and inadequate care creates a recipe for high mortality rates during epidemics in regions with unstable malaria. Overwhelmed health care facilities also result in underreporting of cases, and subsequently the true burden of these outbreaks in unstable transmission regions is unknown (Kiszewski and Teklehaimanot 2004).

Epidemics of leishmaniasis, Chagas, dengue, and St. Louis encephalitis also occur. The mechanisms that are thought to stimulate these outbreaks are similar for these different diseases. Migrations of people from non-endemic regions to endemic regions often result in outbreaks of malaria because lack of exposure to malaria in

these individuals makes them highly susceptible to clinical manifestations of the disease (Kiszewski and Teklehaimanot 2004). Similarly, movement of non-immune individuals in southern Sudan as a consequence of civil war contributed to a series of devastating epidemics of visceral leishmaniasis from 1984 to 1994 (Seaman et al. 1996). Changes in the environment that enhance transmission potential, such as changes in climate and landscape, as well as the pullback of control programs and increased vector and pathogen resistance, can also prime a region for malaria epidemics (Kiszewski and Teklehaimanot 2004). The same mechanisms produce epidemics in several other vector-borne diseases, including those discussed here with the possible exception of Chagas disease (Gubler 1998). Microepidemics of Chagas disease are thought to be due to orally transmitted Chagas resulting from contaminated food (Prata 2000). Population growth and unplanned urbanization have also contributed to epidemic disease as humans continue to encroach on environments where vector-borne diseases are more readily transmitted (Gubler 1998, Gubler et al. 2002, Jeronimo et al. 1994). While each vector-borne disease confers different immunities in their hosts, it is likely that outbreaks of these diseases pose a similar burden on public health systems to that of epidemic malaria in unstable transmission regions. Consequently, finding means to better understand various vector-borne diseases in unstable settings is an important issue.

Autonomous ordinary differential equations are frequently used for endemic vector-borne diseases, however, other types of differential equation models may be more appropriate for modeling disease in unstable transmission settings. In particular, incorporating delays which occur naturally in vector-borne diseases by expressing the problem as a system of delay-differential equations (DDEs) can result in solutions to the system that exhibit sustained or transient oscillations, as well as more complicated chaotic behavior. Mathematicians have included seasonality in ordinary differential equation models for disease to reflect intra-annual fluctuations that are common in diseases spread by vectors. However, case data for malaria indicates that transmission can show inter-annual fluctuations with a relatively stable period, suggesting that there may be an intrinsic mechanism driving these oscillations. In the following section, we present a simple model for vector-borne disease transmission and extend this model to include different delays that arise naturally in vector-borne disease transmission and give rise to complex dynamics.

2 Models of Vector-Borne Diseases with Delays

Ordinary differential equation (ODE) models of vector-borne diseases have a long history. Following his discovery in the late 19th century that female *Anopheles* mosquitoes are the vector responsible for malaria transmission (McKenzie and Samba 2004), Ronald Ross developed the first model of malaria in 1911 (Ross 1911). This model was later improved on by G. Macdonald in the 1950s. Ever since, the Ross-Macdonald type models have been successfully used to guide health officials in choosing and implementing control strategies to restrict the impact of many vector-borne diseases. Analysis of the Ross-Macdonald model for malaria transmission

suggested that imagicides would be a more effective means of vector control than larvicides (Koella 1991), the vector population does not need to be exterminated but simply reduced below a key threshold, and a multi-faceted approach to malaria control would be more effective than any single type of intervention (McKenzie and Samba 2004). People began to build upon the original Ross-Macdonald model, introducing additional complexities such as human immunity. Such a model was developed and confronted with data in the Garki project in Nigeria (McKenzie and Samba 2004), a project devoted to understanding the epidemiology of malaria and determining effective control interventions in West Africa (Molineaux and Gramiccia 1982). Just as introducing human immunity into the Ross-Macdonald model was a natural extension in the Garki project, incorporating delays is another intuitive way to extend the original model. In the following section, we first introduce a simple Ross-Macdonald type ODE model of a vector-borne disease without immunity. We reduce the model to a classical two equation model. Then, we consider several modifications of the vector-borne ODE models by introducing delay into them. Although the epidemiology of each vector-borne disease is unique, the models presented in the following section provide a framework that captures the features common across many vector-borne diseases as well as a framework from which we can build models tailored to a particular disease.

2.1 ODE Models of Vector-Borne Diseases

Transmission in vector-borne diseases involves at least two species, the vector and the host. Since most vectors once infected do not recover, the simplest model for the vector is an SI model. Let us denote the susceptible vectors by S_v and the infected vectors by I_v. A susceptible vector becomes infected upon biting an infected human I_H with a biting rate a and probability of transmission of the disease given by p. The dynamical system that describes the vector is given by the following differential equations:

$$\begin{aligned} S_v' &= \Lambda_v - paS_vI_H - \mu S_v \\ I_v' &= paS_vI_H - \mu I_v \end{aligned} \tag{2.1}$$

Here, Λ_v is the birth rate of the vectors, and μ is the death rate of the vectors. Since the vectors, such as the mosquito, usually have a very short life-cycle, demography should be included. The total vector population size $N_v = S_v + I_v$ is then given by the constrained logistic equation $N_v' = \Lambda_v - \mu N_v$ whose solution can be obtained in explicit form. Since $N_v(t)$ is essentially a given function of t, we may express the number of susceptible vectors in terms of infected vectors $S_v = N_v - I_v$ and replace it in the second equation of system (2.1), thus reducing the two-dimensional vector system to one equation

$$I_v' = pa(N_v(t) - I_v)I_H - \mu I_v \tag{2.2}$$

Now, we turn to the system for the humans. Although humans usually recover from an infection, for most vector-borne diseases recovery is not permanent and the recovered individual can become re-infected. As a starting point, we model the transmission of a vector-borne disease in humans with an SIS model. Some of the vector-borne diseases, such as chikungunya, occur as outbreaks, and in this case, omitting births and deaths for humans is acceptable. Other vector-borne diseases, such as malaria, are endemic and inclusion of demography in the human portion of the model is necessary. We begin with the simplest host model – an SIS model without demography. However, involving host's demography will result in the same limiting system that we will study, so we lose no generality by assuming that there is no demography in the host population. Susceptible hosts in class S_H become infected when bitten by an infectious vector. If we assume that infected vectors bite at the same rate as susceptible vectors, namely a, with q denoting the probability of transmission, then the model takes the form.

$$\begin{aligned} S_H' &= -qaS_H I_v + \alpha I_H \\ I_H' &= qaS_H I_v - \alpha I_H \end{aligned} \tag{2.3}$$

where α is the recovery rate. The total host population size N_H is constant. We can reduce the host system by replacing the susceptible hosts S_H with $S_H = N_H - I_H$ in the second equation. The system above (2.3) reduces to the following equation

$$I_H' = qa(N_H - I_H)I_v - \alpha I_H \tag{2.4}$$

The system for the infected vectors and infected humans becomes

$$\begin{aligned} I_v' &= pa(N_v(t) - I_v)I_H - \mu I_v \\ I_H' &= qa(N_H - I_H)I_v - \alpha I_H \end{aligned} \tag{2.5}$$

The right-hand side of this system depends on the unknown dependent variables I_v and I_H, and the known function of time $N_v(t)$. This makes the right-hand side explicitly dependent on time, and the model **non-autonomous**. However, system (2.5) depends on time only through the function $N_v(t)$ which has a limit as time goes to infinity, namely,

$$N_v(t) \to \frac{\Lambda_v}{\mu} = N_v.$$

Since all solutions of the original system are bounded, results on asymptotically autonomous systems (Thieme 1993) allow us to replace system (2.5) with the following limiting system

$$\begin{aligned} I_v' &= pa(N_v - I_v)I_H - \mu I_v \\ I_H' &= qa(N_H - I_H)I_v - \alpha I_H \end{aligned} \tag{2.6}$$

The limiting system (2.6) is an autonomous system, which is easier to work with. It only contains as dynamic variables the number of infected humans and the number of infected mosquitos. Sometimes a rescaled version of the system is considered where the proportions of infected humans and the proportion of infected mosquitoes are incorporated. In malaria, for instance, it is known from studies that only a small proportion of the mosquitoes are actually infected. The fraction of infected mosquitoes varies around 1% (Bockarie and Dagoro 2006).

System (2.6) has been thoroughly analyzed. To state the results on the global behavior we define the reproduction number of the vector-borne disease. Transmission of vector-borne diseases involves two transmission cycles, namely host to vector and vector to host, and each of these transmission processes may be characterized by its own disease reproduction number. These two numbers may be combined to form a single dimensionless number that indicates whether or not, and to some extent how seriously, the vector-host system is open to invasion by the parasite. The Kermack-McKendrick-Macdonald approach places one infected human in a population of susceptible vectors; this will result in $\mathcal{R}_H$ secondary infected vectors. Similarly, placing one infected vector in a population of susceptible humans, will produce $\mathcal{R}_M$ infected humans, where

$$\mathcal{R}_H = \frac{paN_v}{\alpha}, \qquad \mathcal{R}_M = \frac{qaN_H}{\mu}.$$

To connect these definitions to the mathematical expressions for $\mathcal{R}_H$ and $\mathcal{R}_M$, consider the incidence term in the equation for the vectors $pa(N_v - I_v)I_H$ which gives the number of secondary infections of vectors I_H infected hosts will produce per unit of time. Then, one infected host will produce paN_v infected vectors in an entirely susceptible vector population per unit of time. One infected host is infectious for $1/\alpha$ time units, hence we obtain $\mathcal{R}_H$. Similar reasoning leads to the expression for $\mathcal{R}_M$. To account for the secondary **host** infections that one infected host will produce, we notice that one infected host will produce $\mathcal{R}_H$ infected vectors, each of which will produce $\mathcal{R}_M$ infected hosts, giving

$$\mathcal{R}_0 = \mathcal{R}_H \mathcal{R}_M$$

secondary host infections. This expression gives the classical reproduction number of vector-borne diseases. The reproduction numbers of some of the vector-borne diseases with human host are given in Table 2.1. These reproduction numbers are defined as the number of secondary infections that one infected individual will produce in an entirely susceptible population.

Table 2.1 Vector-borne diseases and their reproduction numbers

Disease	$\mathcal{R}_0$	Region	Years	References
Malaria	1-3000	Africa	-	(Smith et al. 2007)
Dengue	2.0-3.09	Colima, Mexico	2002	(Chowell et al. 2007)
Dengue	8.0	Bandung, Indonesia	2003-2007	(Supriatna 2009)
Chagas disease	1.25	Brazil	2006	(Massad 2008)
Yellow Fever	2.38-3.59	New Orleans	1878	(Curtis et al. 2007)
Chikungunya	0.35-2.3	Reunion Island	2005-2006	(Dumont et al. 2008)
CCHF[a]	2.18	-	-	(Matser et al. 2009)
TBE[b]	1.58	-	-	(Matser et al. 2009)

[a]Crimean-Congo hemorrhagic fever (CCHF)
[b]Tick-borne encephalitis (TBE)

The model (2.6) has two equilibria: a disease free equilibrium $\varepsilon_0 = (0,0)$, and an endemic equilibrium, $\varepsilon^* = (I_v^*, I_H^*)$ where

$$I_H^* = N_H \frac{\mathcal{R}_0 - 1}{\frac{paN_H}{\mu} + \mathcal{R}_0}, \qquad I_v^* = N_v \frac{\mathcal{R}_0 - 1}{\frac{qaN_v}{\alpha} + \mathcal{R}_0}. \tag{2.7}$$

From these expressions it is clear that the endemic equilibrium exists and is positive if and only if $\mathcal{R}_0 > 1$. Furthermore, it can be established that the disease-free equilibrium is globally asymptotically stable if $\mathcal{R}_0 < 1$ and unstable if $\mathcal{R}_0 > 1$. In addition, the endemic equilibrium is locally and globally stable, whenever it exists. This means that *all* solutions that start from positive initial conditions converge to the endemic equilibrium.

2.2 Models of Vector-Borne Diseases with Delays

Delay differential equations differ from ordinary differential equations in that the derivative at any time depends on the solution at prior times. The simplest constant delay equations have the form

$$x'(t) = F(t, x(t), x(t-\tau_1), x(t-\tau_2), \ldots, x(t-\tau_k))$$

where the time delays τ_j are positive constants. Additional information is required to specify a system of delay differential equations. Because the derivative in the equation above depends on the solution at the previous time $t-\tau_j$, it is necessary to provide an initial history function, or a vector of functions, to specify the value of the solution before time $t=0$.

Interest in such systems arises when traditional pointwise modeling assumptions are replaced by dependence of the rate of change on the prior population numbers.

As mentioned in the introduction, delays occur naturally in vector-borne diseases because steps in the development of the vector and the pathogen take a significant amount of time, particularly compared to the lifespan of the vector. This makes delay differential equations a natural choice for modeling vector-borne diseases. Three typical time delays have so far been incorporated in mathematical models of vector-borne diseases. These are:

2.2.1 Delays related to the extrinsic incubation period

When the pathogen enters the body of the vector, some time elapses before the vector becomes infectious. This time period is called the **extrinsic incubation period**. Inclusion of the extrinsic incubation period in the dynamics of the vector is particularly important as the length of that period is often of duration comparable to the mean lifespan of the vector. For instance, the extrinsic incubation period of *Plasmodium* species that cause malaria is about two weeks, while on average, a female mosquito is known to live anywhere between 15 to 100 days. These incubation periods tend to be shorter at higher temperatures and longer at lower temperatures for several pathogens, including Plasmodium parasites, dengue viruses, and the St. Louis encephalitis virus (Ruan et al. 2009, Patz 2000). The fact that vectors may or may not survive the extrinsic incubation period affects significantly the dynamics of the infectious disease. This makes imperative the inclusion of the extrinsic incubation period as a delay in the vector-host epidemic models. Furthermore, delay models of this type include the probability that the vector survives the extrinsic incubation period.

To incorporate the delay caused by the extrinsic incubation period, we modify equations (2.6). We include the delay in the incidence term, as well as the probability that the vector survives that delay. The vectors that become infectious at time t were infected at time $t-\tau$ where τ is the delay induced by the extrinsic incubation period. In practical terms τ is, in fact, given by the length of the extrinsic period. For instance, in malaria, since the length of the extrinsic incubation period is about two weeks, then $\tau \approx 0.5$ months. The number of vectors becoming infectious is given by the number of vectors infected $t-\tau$ units ago: $pa(N_v - I_v(t-\tau))I_H(t-\tau)$ discounted by the probability of survival of the vector, given by $e^{-\mu\tau}$. Including the probability of survival of the vector is important. In malaria, for instance, only 40% of the vectors survive the extrinsic incubation period (Cox et al. 1999), even in optimal environmental conditions.

With the inclusion of the delay corresponding to the extrinsic incubation period, model (2.6) becomes:

$$\begin{aligned} I_v' &= pae^{-\mu\tau}(N_v - I_v(t-\tau))I_H(t-\tau) - \mu I_v \\ I_H' &= qa(N_H - I_H)I_v - \alpha I_H \end{aligned} \tag{2.8}$$

The first equation in the system above is a differential-delay equation where the unknown functions depend on the delay. In order to solve the system above, we need to know $I_v(\theta)$ and $I_H(\theta)$ for $\theta \in [-\tau, 0]$.

2.2.2 Delays related to the intrinsic incubation period

Besides the incubation period in the vector, vector-borne pathogens also have an incubation period within the host. This incubation period is called the **intrinsic incubation period**. Although the intrinsic incubation period is much shorter relative to the host lifespan, it is often customary to include it as a delay in the vector-host model. For instance, the intrinsic incubation period of malaria is 6 to 25 days, while the average lifespan of humans is roughly 70 years. Although the probability that the host survives the intrinsic incubation period is very large, this probability is still included in vector-borne disease models.

To incorporate the delay caused by the intrinsic incubation period, we modify again equations (2.6). We include the delay in the incidence term, as well as the probability the host survives that delay. Hosts that become infectious at time t were infected at time $t-\tau$, where τ is the delay induced by the intrinsic incubation period. The number of those becoming infectious is given by the number of those infected $t-\tau$ units ago: $qa(N_H-I_H(t-\tau))I_v(t-\tau)$, discounted by the probability of survival of the host as infectious, given by $e^{-\alpha\tau}$.

The model (2.6), modified by incorporating delay within the host, becomes:

$$\begin{aligned} I_v' &= pa(N_v - I_v)I_H - \mu I_v \\ I_H' &= qae^{-\alpha\tau}(N_H - I_H(t-\tau))I_v(t-\tau) - \alpha I_H \end{aligned} \tag{2.9}$$

The second equation in the system above is a differential-delay equation where the unknown functions depend on the delay. We need to know $I_v(\theta)$ and $I_H(\theta)$ where $\theta \in [-\tau, 0]$ in order to solve the system above.

Inclusion of delay in response to the intrinsic incubation period is of less importance as the host has a relatively high probability of surviving the incubation period once infected, and subsequently becoming infectious. For this reason, models as the one above are typically not considered. However, models that involve two delays, one to include the extrinsic incubation period, and another to include the intrinsic incubation period, are of particular interest. We include here the Ross-Macdonald model with two delays, introduced by Ruan et al. (2009). If $\tau_1 > 0$ is the delay caused by the extrinsic incubation period and τ_2 is the delay caused by the intrinsic incubation period, then the combination of model (2.8) and model (2.9) results in the following differential-delay model with two delays:

$$\begin{aligned} I_v' &= pae^{-\mu\tau_1}(N_v - I_v(t-\tau_1))I_H(t-\tau_1) - \mu I_v \\ I_H' &= qae^{-\alpha\tau_2}(N_H - I_H(t-\tau_2))I_v(t-\tau_2) - \alpha I_H \end{aligned} \tag{2.10}$$

The above model was considered by Ruan et al. (2009) who established the following results. The reproduction number of the model (2.10) is given by

$$\mathcal{R}_0 = \frac{pqa^2 N_v N_H e^{-\mu\tau_1} e^{-\alpha\tau_2}}{\mu\alpha},$$

which can also be interpreted as the product of the human and vector reproduction numbers. When $\mathcal{R}_0 < 1$, then the system has a unique disease-free equilibrium $\mathcal{E}_0 = (0,0)$ which is locally stable. If $\mathcal{R}_0 > 1$, then the system also has an endemic equilibrium $\mathcal{R}^* = (I_v^*, I_H^*)$ and the disease-free equilibrium is unstable. Furthermore, when $\tau_1 = 0$, there exists τ_2^* such that the endemic equilibrium is locally asymptotically stable for $\tau_2 \in [0, \tau_2^*)$. Finally, for $\tau_2 \in [0, \tau_2^*)$ there exists $\tau_1^*(\tau_2^*)$ such that the endemic equilibrium is locally asymptotically stable for $\tau_1 \in [0, \tau_1^*)$ and $\tau_2 \in [0, \tau_2^*)$. Ruan *et al.* do not consider the special but important case when the extrinsic incubation period is taken into account ($\tau_1 \neq 0$) while the intrinsic incubation period is not ($\tau_2 = 0$).

It is important to note that delay equations can be simulated just as the ordinary differential equations using computer algebra systems such as MATLAB, Mathematica and others. In particular, using such computer systems delay differential equations can be fitted to data – both prevalence and incidence data. When fitted to human incidence data, the human incidence term $qae^{-\alpha\tau 2}(N_H - I_H(t-\tau_2))I_v(t-\tau_2)$ has to be fitted to the given data at time t.

2.2.3 Delays related to the maturation period of the vector

The last source of delays in vector-borne models comes from the adaptive maturation delays of the vector. Many vectors, which are arthropods, undergo several life stages before they reach adulthood and are able to transmit the disease. For instance, a mosquito's life-cycle consists of three successive juvenile phases (egg, larva, pupa) before reaching the adult phase. It usually takes about 1-2 weeks before mosquitoes mature to adulthood, a time frame which is large relative to the average lifespan of the mosquito. To account for this delay, delay-differential equation models with delay in recruitment are composed. Such models have been previously considered by Fan et al. (2010) in the discussion of the impact on dynamics of the mosquito-borne pathogen West Nile Virus, and by Ngwa et al. (2010) in the discussion of a model, focused on the vector, with maturation delays. Prolonged developmental times are also experienced by other vectors, such as triatomines (Triatominae, Reduviidae), the vectors of Chagas disease Ngwa et al. (2010).

To develop a vector-borne disease model with maturation delays, we need to use a baseline ODE model that incorporates recruitment of the vector. Hence, model (2.6) is not appropriate. We need to go back to model (2.1). Development of juvenile stages of vectors is density dependent and it is best modeled through a Ricker's type function as a recruitment rate into the population of adult vectors. If we denote the maturation delay by τ, then the total number of vectors that produce offsprings at time $t-\tau$ is $N_V(t-\tau)$. Suppose d_v is the death rate of juvenile vectors. Then, the probability of a juvenile vector surviving the juvenile stages and becoming an adult is $e^{-d_V\tau}$. The Ricker density dependent model assumes that the per capita birth rate declines exponentially with population size, so a term of the form $e^{-\rho N_V(t-\tau)}$ is included, where $1/\rho$ is the size of the vector population at which progeny production is maximized for a given total adult population size. Finally, r is the maximum per capita per unit of time vector progeny production rate. We replace the constant recruitment

rate of the vector in model (2.1) with the recruitment rate $rN_V(t-\tau)e^{-\rho N_V(t-\tau)}e^{-d_V\tau}$. The model with maturation delay of the vector becomes

$$\begin{aligned} S_v' &= rN_V(t-\tau)e^{-\rho N_v(t-\tau)}e^{-d_v\tau} - paS_vI_H - vS_v \\ I_v' &= paS_vI_H - vI_v \\ S_H' &= -qaS_HI_v + \alpha I_H \\ I_H' &= qaS_HI_v - \alpha I_H \end{aligned} \tag{2.11}$$

The total population size of the vector in this model is given by the following delay-differential equation:

$$N_v' = rN_V(t-\tau)e^{-\rho N_v(t-\tau)}e^{-d_v\tau} - vN_v. \tag{2.12}$$

The equation for the total vector population size (2.12) has been completely analyzed (Cook et al. 1999), and oscillations in that model have been found. Because the total population size of the vector is not necessarily asymptotically constant, the equation for the susceptible vectors cannot be eliminated from the above model. However, since the total population size for the human host remains constant, the susceptible human host population can still be removed.

3 Unstable Dynamics of Vector-Borne Diseases and Delay Differential Equation Models

Vector-borne diseases exhibit different patterns of occurrence. Parasitic and bacterial diseases, such as malaria and Lyme disease, tend to produce a high disease incidence that is not typically confounded with major epidemics. An exception to this rule is plague, a bacterial disease that does cause outbreaks. In contrast, many vector viral diseases, such as Yellow fever, dengue, Japanese encephalitis, and chikungunya commonly cause major epidemics.

3.1 Unstable Dynamics of Vector-Borne Diseases: Malaria as a Case Study

Even though the dynamics of malaria, one of the most prominent and deadly vector-borne diseases, is typically stable and persistent, exceptions to this observation exist as we illustrated in section 2.1.2. These exceptions have serious implications for modeling, response, and control of malaria. Unstable dynamics of malaria can occur in two distinct regimes:

1. relatively low baseline prevalence with occasional major outbreaks;
2. nearly oscillatory behavior where high prevalence follows low prevalence in consecutive years.

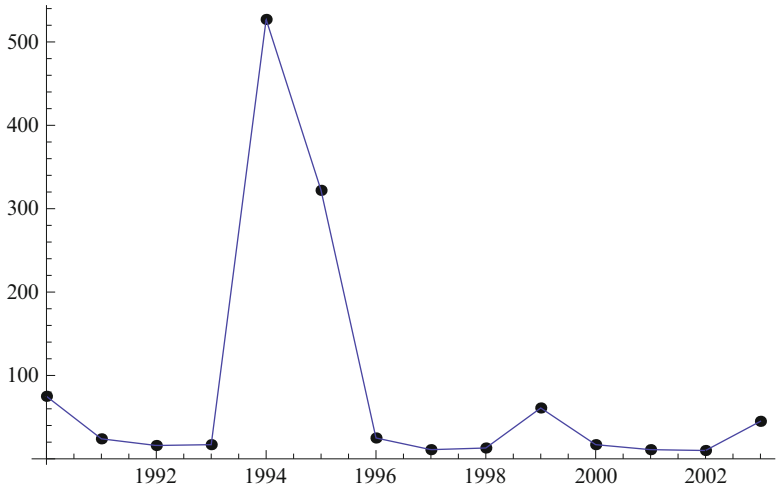

Fig. 2.1 Number of malaria cases in Egypt for the years 1990–2003. The data exhibit background oscillatory dynamics with an outbreak in 1994. Data taken from (WHO).

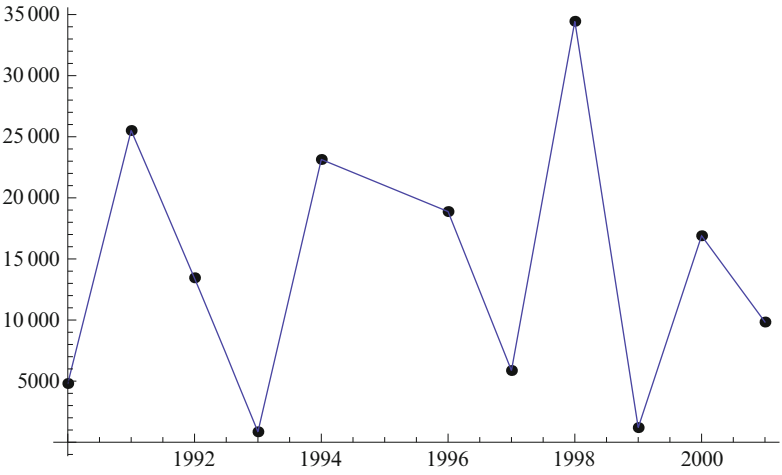

Fig. 2.2 Number of *P. falciparum* cases in Haiti for the years 1990–2001. The data exhibit clear oscillatory dynamics. Data taken from (WHO).

The disease dynamics of several countries, including Botswana, Egypt, Iraq, Kyrgyzstan and Turkmenistan, have exhibited the first type of instability since 1990. The case of Egypt is illustrated in Fig. 2.1 where a major outbreak occurred in 1994 and resulted in nearly 10 times the usual number of cases. Brazil, and particularly Haiti in the period 1990-2000, are examples of the second type of dynamics where the malaria prevalence oscillates between high and low with a relatively stable median. The number of cases in Haiti is given in Fig. 2.2.

Major outbreaks or epidemics of malaria occur primarily in regions where the overall transmissibility of the disease is low. The unstable nature of malaria in such regions, and of other vector-borne diseases, present serious clinical threat to the populations of the affected areas. Inter-epidemic periods of very low transmissibility, particularly when long, allow for the immunity in the population to wane. Thus, during an outbreak or epidemic, young children are at higher risk of contracting malaria, while older children and adults are much more vulnerable to serious complications of the disease compared with stable transmission settings. The randomness of the outbreaks has a serious detrimental impact on the ability to predict, prepare for, and control the outbreak. Consequently, the outbreaks present a burden to the health care system of epidemic-prone countries.

Nearly oscillatory behavior, although more predictable, requires significant flexibility and adaptability of the response network. Similar difficulties arise in the control of malaria in such areas. The reasons for the inter-annual cycles of malaria, exhibited in such areas, are not completely understood, which complicates the efficient control of the disease in years of higher prevalence.

3.2 Capturing Oscillatory Dynamics and Chaos with Delay Models

Traditionally, vector borne diseases have been modeled by ordinary differential equations. The delays introduced by the incubation and maturation periods can be included in ODE models by incorporating additional stages in the model. For instance, the incubation periods can be modeled via exposed compartments in the vector and/or the host systems. Such models have been considered in (Chitnis et al. 2006). However, these compartmental ODE systems are only an adequate modeling tool when the disease exhibits stable dynamics as they typically predict convergence to an equilibrium. Ordinary differential equations in general display a low potential for complex dynamics. Oscillatory dynamics in ODEs occurs in two or higher dimensional systems. Chaos can only be obtained from three or higher dimensional systems. Yet, even high dimensional ODE models tend to have globally stable equilibria. In contrast, delay-differential equations can exhibit complex dynamics – oscillations and chaos – even in one dimensional models. Moreover, delay-differential models of vector-borne diseases, unlike their ODE counterparts, are capable of showing such complex dynamics. This makes them a better modeling tool for unstably transmitted vector-borne diseases. The idea that vector-borne disease models with delay can model oscillatory dynamics is not new. Several articles suggest that delay models of vector-borne diseases can exhibit oscillatory behavior (Thieme 1993). Consider a model of a vector-borne disease with permanent immunity and delay. They show that the endemic equilibrium can be destabilized via Hopf bifurcation. More recently Saker (2010) established the presence of Hopf bifurcation in the vector-host model with two delays (2.10). Other authors have

also found oscillations in delay-differential equation models of vector-borne diseases (Hancock and Goodfray 2007, Tang 2007, Wei et al. 2008).

In what follows we show that delay equations, even a simple single delay equation, are capable of displaying oscillations and chaos. To obtain this single equation, we begin from the delay model with two delays (2.10). The single delay equation that we derive is suitable to model malaria, and other vector borne diseases, where the extrinsic and intrinsic incubation periods are nearly equal in duration.

3.2.1 Reducing the Delay Model to a Single Equation

Biologists often use various methods to reduce the dimension of a system describing vector-borne disease. The newly obtained system does not necessarily have the same dynamical behavior as the original one but it is still useful in obtaining initial insights into the disease dynamics.

Justification for the reduction in dimension is typically based on the assumption that the lifespan of the vector is much shorter than the duration of infectiousness of the humans, that is, we assume that $\mu >> \alpha$ and this leads to much faster equilibration of the dynamics of the vector population compared with the host population. This assumption is common for vector- borne diseases transmitted by mosquitoes, such as malaria (Chiyaka et al. 2010). Furthermore, we assume that the intrinsic incubation period is approximately equal to the extrinsic incubation period, that is $\tau_1 = \tau_2 = \tau$. This is certainly the case in malaria where the incubation period in the humans typically lasts between 10 days and four weeks. The extrinsic period is often temperature-dependent but lasts 10-18 days. If we assume that the two incubation periods are the same, the model with two delays (2.10) becomes:

$$\begin{aligned} I_v' &= pae^{-\mu\tau}(N_v - I_v(t-\tau))I_H(t-\tau) - \mu I_v \\ I_H' &= qae^{-\alpha\tau}(N_H - I_H(t-\tau))I_v(t-\tau) - \alpha I_H \end{aligned} \tag{2.13}$$

Furthermore, since the vector dynamics has reached equilibrium, we have $I_v' = 0$. At equilibrium, the population numbers at time t and $t-\tau$ are approximately the same. Hence, from the first equation we have

$$I_v(t-\tau) = \frac{pae^{-\mu\tau}N_v I_H(t-\tau)}{pae^{-\mu\tau}I_H(t-\tau) + \mu}.$$

Substituting I_v in the second equation, we obtain the following single delay equation for the dynamics of the humans:

$$I_H' = \frac{pa^2 qe^{-\alpha\tau}e^{-\mu\tau}N_v I_H(t-\tau)}{pae^{-\mu\tau}I_H(t-\tau) + \mu}(N_H - I_H(t-\tau)) - \alpha I_H \tag{2.14}$$

It is helpful to normalize this equation by setting $x = I_H/N_H$. The equation for the proportion of humans infected becomes:

$$x' = \frac{pa^2 qme^{-\alpha\tau}e^{-\mu\tau}x(t-\tau)}{pae^{-\mu\tau}x(t-\tau)+\mu}(1-x(t-\tau)) - \alpha x(t) \tag{2.15}$$

where $m = N_v/N_H$ is the ratio of the number of vectors to the number of humans and aN_H has been replaced again by a.

Delay equations, just like ODEs, have equilibria. The value x^* is an equilibrium of model (2.15) if it satisfies the equation

$$\frac{pa^2 qme^{-\alpha\tau}e^{-\mu\tau}x^*}{pae^{-\mu\tau}x^*+\mu}(1-x^*) - \alpha x^* = 0. \tag{2.16}$$

This equation clearly has the solution $x^* = 0$ which gives the disease-free equilibrium. To investigate the stability of the disease-free equilibrium, we linearize the equation. We look for a solution $x(t) = x^* + y(t)$ where $y(t)$ is the perturbation around the equilibrium, and $x^* = 0$. This means that we have to replace x with y and linearize the nonlinear term. Notice that

$$\frac{1}{pae^{-\mu\tau}x(t-\tau)+\mu} = \frac{1}{\mu(pa/\mu e^{-\mu\tau}y(t-\tau)+1)} \approx \frac{1}{\mu}\left[1 - pa/\mu e^{-\mu\tau}y(t-\tau)\right].$$

Hence, the linearization around the disease-free equilibrium is given by:

$$y' = \frac{pa^2 qme^{-\alpha\tau}e^{-\mu\tau}y(t-\tau)}{\mu} - \alpha y(t).$$

Because we now have a linear system, we look for a solution of the form $y(t) = \overline{y}e^{\lambda t}$, and subsequently obtain the following characteristic equation

$$\lambda + \alpha = \frac{pa^2 qme^{-\alpha\tau}e^{-\mu\tau}e^{-\lambda\tau}}{\mu}.$$

The above equation is a **transcendental equation**, that is an equation containing a transcendental function of λ, namely $e^{\lambda\tau}$. λ can be a real or complex variable. If we think of λ as a real variable, the left-hand side of the above equation is an increasing linear function of λ while the right-hand side is a decreasing function of λ. This equation always has a unique real solution which is positive if and only if $\mathcal{R}_0 > 1$ where we define the reproduction number $\mathcal{R}_0$ to be

$$\mathcal{R}_0 = \frac{pa^2 qme^{-\alpha\tau}e^{-\mu\tau}}{\mu\alpha}. \tag{2.17}$$

So if $\mathcal{R}_0 > 1$, the disease-free equilibrium is unstable. If $\mathcal{R}_0 < 1$, the unique real eigenvalue is negative. We show that all other eigenvalues, which are complex, have negative real parts. Assume we have an eigenvalue $\lambda = b + ci$, where i is the imaginary unit, that has a nonnegative real part, that is $b \geq 0$. Then $|\lambda + \alpha| = \sqrt{(b+\alpha)^2 + c^2} \geq b + \alpha \geq \alpha$. At the same time

$$\begin{aligned} &\left| \frac{pa^2 qme^{-\alpha\tau}e^{-\mu\tau}e^{-\lambda\tau}}{\mu} \right| \\ &= \frac{pa^2 qme^{-\alpha\tau}e^{-\mu\tau}\,|\,e^{-\lambda\tau}\,|}{\mu} \\ &= \frac{pa^2 qme^{-\alpha\tau}e^{-\mu\tau}e^{-b\tau}}{\mu} \\ &\leq \frac{pa^2 qme^{-\alpha\tau}e^{-\mu\tau}}{\mu} \end{aligned} \tag{2.18}$$

which contradicts the fact that $\mathcal{R}_0 < 1$, that is $\alpha > \frac{pa^2 qme^{-\alpha\tau}e^{-\mu\tau}}{\mu}$. Hence, because all the eigenvalues have negative real parts, the disease-free equilibrium is locally asymptotically stable if $\mathcal{R}_0 < 1$. We note that if $\mathcal{R}_0 = 1$, then $\lambda = 0$ is an eigenvalue and we cannot use this argument to make conclusions. We consider again the equation for the equilibria. Canceling x^*, the equation for the equilibria (2.16).

$$\frac{pa^2 qme^{-\alpha\tau}e^{-\mu\tau}}{pae^{-\mu\tau}x^* + \mu}(1 - x^*) - \alpha = 0. \tag{2.19}$$

Multiplying by the denominator, we obtain a linear equation in x^* which can be solved to give the unique endemic equilibrium.

$$x^* = \frac{\mathcal{R}_0 - 1}{pa/\mu e^{-\mu\tau} + \mathcal{R}_0}. \tag{2.20}$$

It is clear from this expression that the endemic equilibrium exists and is positive if and only if $\mathcal{R}_0 > 1$. To investigate the stability of the endemic equilibrium, we linearize around it. Set $x(t) = x^* + y(t)$, where $y(t)$ is the perturbation of the endemic equilibrium. The perturbation y can take positive and negative values. Furthermore,

to simplify the notation, we will denote $Q = pa^2qme^{-\alpha\tau}e^{-\mu\tau}$ and $P = pae^{-\mu\tau}$. Substituting in the delay equation (2.3) we obtain the following equation for the perturbation

$$y'(t) = \frac{Q(x^* + y(t-\tau))}{P(x^* + y(t-\tau)) + \mu}[1 - x^* - y(t-\tau)] - \alpha(x^* + y(t)). \tag{2.21}$$

Taking into account the equation for the equilibrium

$$\frac{Qx^*(1-x^*)}{Px^* + \mu} = \alpha x^* \tag{2.22}$$

and linearizing as in the case of the disease-free equilibrium, we obtain the following equation for the perturbation y:

$$y'(t) = \frac{Q(1-x^*)y(t-\tau)}{Px^* + \mu} - \frac{Qx^*}{Px^* + \mu}\left[\frac{P(1-x^*)y(t-\tau)}{Px^* + \mu} + y(t-\tau)\right] - \alpha y(t). \tag{2.23}$$

This equation can be simplified as follows:

$$y'(t) = \frac{Q(1-x^*)y(t-\tau)}{Px^* + \mu}\left[1 - \frac{Px^*}{Px^* + \mu}\right] - \frac{Qx^*y(t-\tau)}{Px^* + \mu} - \alpha y(t). \tag{2.24}$$

Using the equation for the equilibrium (2.22) and the fact that $\mathcal{R}_0 = Q/(\alpha\mu)$, we obtain the following simplified linearized equation

$$y'(t) = \frac{\alpha\mu}{Px^* + \mu}(1 - \mathcal{R}_0 x^*)y(t-\tau) - \alpha y(t). \tag{2.25}$$

Looking for the exponential solution $y(t) = \overline{y}e^{\lambda t}$, we obtain the following characteristic equation

$$\lambda + \alpha = \frac{\alpha\mu}{Px^* + \mu}(1 - \mathcal{R}_0 x^*)e^{-\lambda\tau}. \tag{2.26}$$

If $\mathcal{R}_0 x^* < 1$, the coefficient in front of the term $e^{-\lambda\tau}$ is positive and smaller than α, which corresponds to the case when $\mathcal{R}_0 < 1$ in the characteristic equation for the disease-free equilibrium. A similar argument can show that all roots of the equation

(2.26) have negative real parts, and the endemic equilibrium is locally asymptotically stable. We summarize these results in the following Theorem:

Theorem 1 *If $\mathcal{R}_0 < 1$ the differential delay equation (2.15) has only the disease-free equilibrium $x^* = 0$ which is locally asymptotically stable. If $\mathcal{R}_0 > 1$ the differential delay equation (2.15) has the disease-free equilibrium and a unique endemic equilibrium x^*. If $\mathcal{R}_0 > 1$ the disease-free equilibrium is unstable. The endemic equilibrium is locally asymptotically stable, if in addition $\mathcal{R}_0 x^* < 1$.*

This Theorem suggests a rather curious conclusion – the endemic equilibrium is stable if $x^* < 1/\mathcal{R}_0$. Since the reproduction number of malaria $\mathcal{R}_0$ is often large, then the equilibrium is stable if the fraction of infected individuals is rather small. This suggests that in countries, like Egypt, where the year to year prevalence is typically very low, outbreaks such as the one that occurred in 1994 may not be possible to capture with this simple single-equation model of malaria and may be a result of stochastic events.

3.2.2 Oscillations and Chaos in the Delay Differential Equation

If $\mathcal{R}_0 x^* > 1$, then the coefficient on the right-hand side of the characteristic equation (2.26) is negative, and the equation can have as principal eigenvalues (eigenvalues with the largest real part) a pair of complex conjugate eigenvalues. However, as a parameter changes, this pair of principal eigenvalues may cross the imaginary axis giving rise to a stable oscillatory solution. At the same time, the principal eigenvalues start having positive real part and the endemic equilibrium becomes unstable. This process that gives rise to a stable oscillatory solution is called **Hopf bifurcation**. The result is valid for ODEs and delay-differential equations. For differential delay equations, it is given in the Hopf bifurcation Theorem below:

Theorem 2 *Consider the differential delay equation*

$$x'(t) = F(x(t), x(t-\tau_1), \ldots, x(t-\tau_{n-1}), \mu) \tag{2.27}$$

where μ is a parameter. If:

1. *F is analytic in x and μ in a neighborhood of $(\mathbf{0}, 0)$ in $\Re^n \times \Re$.*

- *$F(\mathbf{0}, \mu) = 0$ for μ in an open interval containing 0, and $x(t) = 0$ is an isolated stationary solution of (2.27).*
- *The characteristic equation of (2.27) has a pair of complex conjugate eigenvalues λ and $\bar{\lambda}$ such that $\lambda(\mu) = b(\mu) + i\omega(\mu)$ where $\omega(0) = \omega_0 > 0$, $b(0) = 0$ and $b'(0) \neq 0$.*
- *The remaining eigenvalues of the characteristic equation have strictly negative real parts.*

Then, the differential delay equation (2.27) has a family of Hopf periodic solutions.

One can apply Theorem 2.3.2 to show rigorously that Hopf bifurcation occurs in equation (2.15). Instead, we will build a specific numerical example of such an

oscillatory solution. To find sustained oscillations in equation (2.15), we need to find values of the parameters for which such oscillations occur. We begin from the characteristic equation (2.26), which we simplify further, and write as

$$\lambda + \alpha = \rho e^{-\lambda\tau} \tag{2.28}$$

where $\rho = \dfrac{\alpha\mu}{Px^* + \mu}(1 - \mathcal{R}_0 x^*)$. We recall that we have assumed that $\rho < 0$. Let $\lambda = b + i\omega$. We separate the real and the imaginary part:

$$\begin{aligned} b + \alpha &= \rho e^{-b\tau} \cos[\omega\tau] \\ \omega &= \rho e^{-b\tau} \sin[\omega\tau]. \end{aligned} \tag{2.29}$$

Now we ask the question: Can we find parameters $\alpha > 0$ and $\rho < 0$ such that the system above has positive solution $b > 0$ and $\omega > 0$? We solve in terms of α and ρ

$$\begin{aligned} \alpha &= -b + \omega \cot[\omega\tau] \\ \rho &= \omega e^{b\tau} csc[\omega\tau]. \end{aligned} \tag{2.30}$$

As we have seen earlier, some of the parameters that have physical meaning can be pre-estimated, or at least reasonable biological ranges can be determined for them. In the equations above, we assume values for b and τ and interpret α and ρ as functions of ω. Using a computer algebra system we can make a parametric plot of α and ρ in the (α, ρ)-plane. This plot is shown in Fig. 2.3.

We pick a value for ω, say $\omega = 5.2$. From system (2.29) we obtain the values $\alpha = 2.74768$ and $\rho = -5.94514$. The value of α corresponds to an infectious period of $1/2.74768 = 0.3639$ years which is a reasonable duration for *Plasmodium falciparum* malaria. Now we have to assume values for the remaining parameters, so that the combined value of ρ is as given. We assume the value $\mu = 12$, which gives a vector lifespan of one month. This duration is a realistic estimate for a mosquito's lifespan. Furthermore, we have to find Q and P so that the following system holds

$$\begin{aligned} \frac{Q(1 - x^*)}{Px^* + \mu} &= \alpha \\ \frac{\mu\alpha(1 - \mathcal{R}_0 x^*)}{Px^* + \mu} &= \rho. \end{aligned} \tag{2.31}$$

Dividing these two equations we have

$$\frac{\mathcal{R}_0(1 - x^*)}{1 - \mathcal{R}_0 x^*} = \frac{\alpha}{\rho}.$$

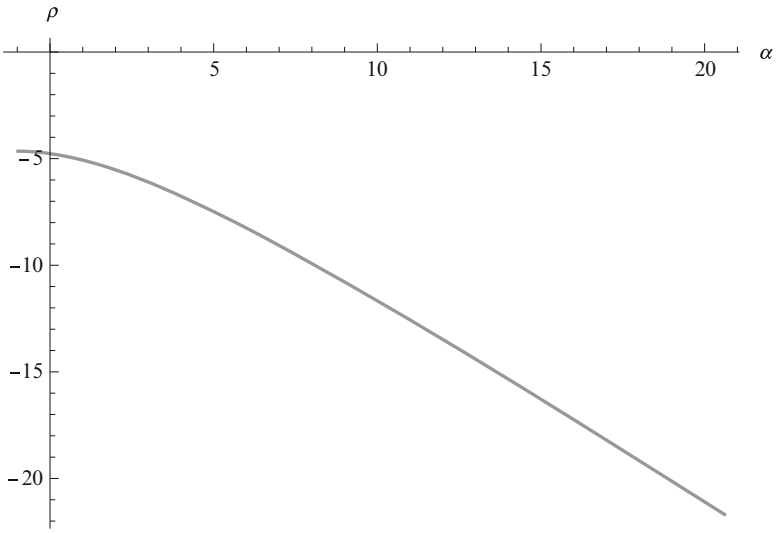

Fig. 2.3 Parametric plot of α and ρ in the (α, ρ)-plane as given by equations (2.18). The values of b and τ are taken as follow: $b=0.01$, $\tau=1$. The value of τ which is equal to one year is rather high for *Plasmodium falciparum* malaria. The plot is made for $4.5 \leq \omega \leq 6$.

From here, assuming a value of $R_0 x^*$, we can compute R_0 as

$$\mathcal{R}_0 = \mathcal{R}_0 x^* + \frac{\alpha}{\rho}(1 - \mathcal{R}_0 x^*).$$

If we take $\mathcal{R}_0 x^* = 5$, then $\mathcal{R}_0 = 6.84869$. From here we can compute $x^* = 0.73$. Finally, $Q = \mathcal{R}_0 \alpha \mu = 225.816$. From the second equation in system (2.31) we determine $P = 13.9498$. With these parameters we plot the solution of equation (2.15) in Fig. 2.4.

The trajectory in Fig. 2.4 suggests that the endemic equilibrium is indeed unstable. However, the trajectory is not periodic. It is *aperiodic*, suggesting the presence of **chaos** in the model (2.3). What is chaos? There are many definitions of chaos. Perhaps the most useful in biology is the following:

Definition 1 *Chaos* is aperiodic long-term behavior in a deterministic system that exhibits sensitive dependence on initial conditions.

This definition has several components:

1. *Aperiodic long-term behavior* means that there are trajectories which do not settle down to fixed points, periodic orbits, or quasi-periodic orbits as $t \to \infty$. For practical purposes we require that these aperiodic orbits are not too rare.
2. *Deterministic* means that the system has no random or noisy inputs.
3. *Sensitive dependence on initial conditions* means that nearby trajectories separate exponentially fast.

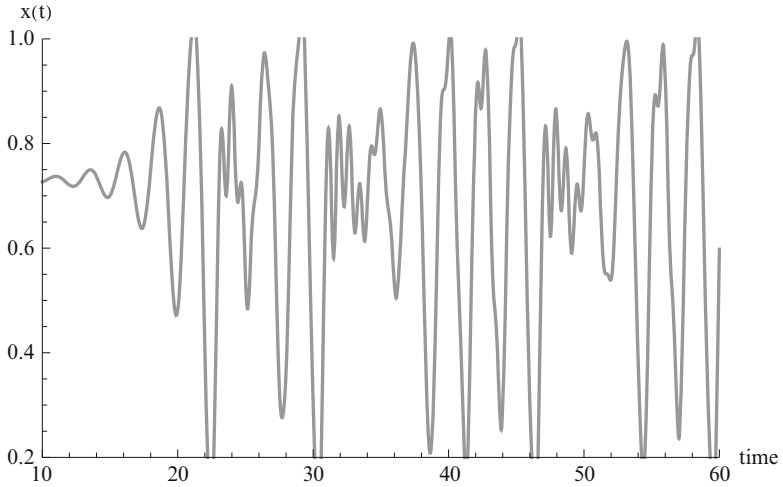

Fig. 2.4 Plot of the solution of equation (2.3) with $P = pae^{-\mu\tau} = 13.9498$, $Q = Pqame^{-\alpha\tau} = 225.816$, $\tau = 1$, $\alpha = 2.74768$, $\mu = 12$ and initial condition $x(0) = 0.73$. The resulting trajectory is aperiodic suggesting presence of chaotic behavior.

From Fig. 2.4 we see that the delay malaria model (2.15) has solutions that are aperiodic, that is their trajectory does not repeat even when we run for a long time. Furthermore, the trajectories exhibit sensitive dependence on initial data. If we start very close to the trajectory above, the two trajectories "coincide" for a certain amount of time, called the *time horizon*, after which the two trajectories completely diverge and one doesn't look like the other. The sensitive dependence is illustrated in Fig. 2.5.

The existence of sensitive dependence on initial conditions in simple but chaotic models means that we have lost the ability to make long-term predictions. We can still make short-term predictions based on chaotic models which are valid for the duration of the time horizon. Chaotic behavior emerges from periodic behavior through a process called period doubling. This sequence of period doubling leading to chaos is often demonstrated on a chaos bifurcation diagram which plots the long-term behavior of the solution with respect to some parameter. Such a chaos bifurcation diagram is plotted in Fig. 2.6. Because chaos emerge from a periodic solution as a result of increase in the delay parameter, this suggests that if we decrease the bifurcation parameter τ, we will obtain a regular periodic solution. This is indeed the case. Fig. 2.7 shows a periodic trajectory produced with the same parameters as above and $\tau = 0.6$.

We see that even first order deterministic delay models can exhibit chaotic behavior and sustained oscillations. This suggests that delay-differential equation models are a suitable tool to produce unstable, oscillatory, nearly oscillatory or chaotic dynamics in vector-borne diseases.

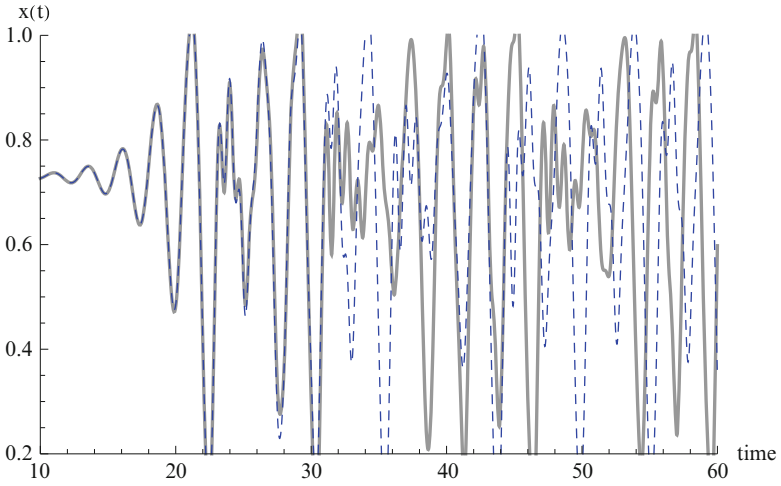

Fig. 2.5 Plot of two solutions of equation (2.3) with $P = pae^{-\mu\tau} = 13.9498$, $Q = Pqame^{-\alpha\tau} = 225.816$, $\tau = 1$, $\alpha = 2.74768$, $\mu = 12$ and initial conditions $x_1(0) = 0.73$ and $x_2(0) = 0.730001$. The two close trajectories coincide for a while and then diverge suggesting sensitive dependence on the initial conditions.

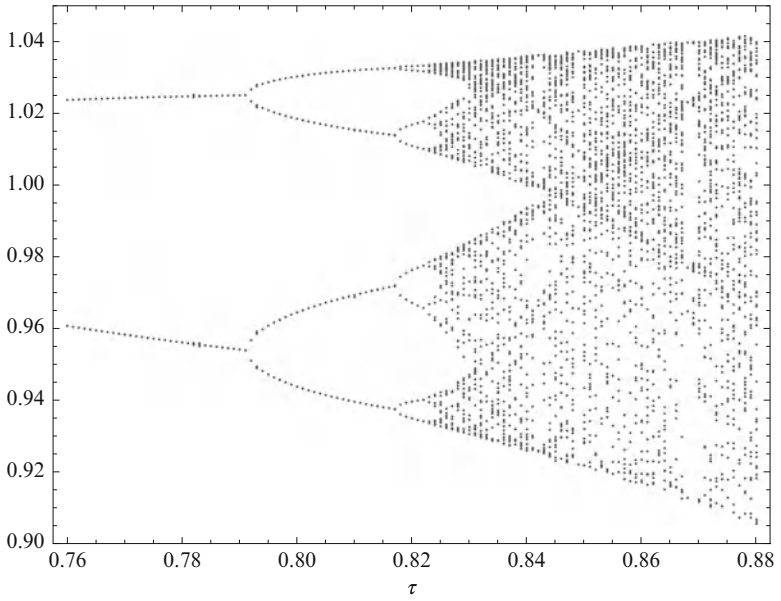

Fig. 2.6 Plot of the chaos bifurcation diagram with $P = pae^{-\mu\tau} = 13.9498$, $Q = Pqame^{-\alpha\tau} = 225.816$, $\alpha = 2.74768$, $\mu = 12$ and initial condition $x(0) = 0.73$. The delay parameter τ is a bifurcation parameter. Long-term behavior of x is plotted on the y axis.

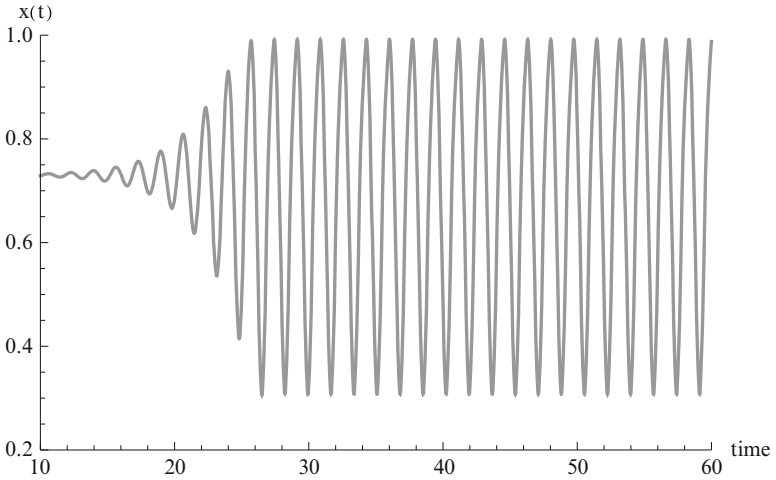

Fig. 2.7 Plot of a periodic solution of equation (2.3) with $P = pae^{-\mu\tau} = 13.9498$, $Q = Pqame^{-\alpha\tau} = 225.816$, $\tau = 0.6$, $\alpha = 2.74768$, $\mu = 12$ and initial condition $x(0) = 0.73$.

3.3 Delay-Differential Equations as a Modeling Tool for Intrinsic Drivers of Instabilities in Vector-BorneDiseases

The main question that needs to be addressed is: How should we model malaria and other vector-borne diseases so that we can capture the instabilities in the dynamics? There are three possibilities that may be used to model and explain unstable outbreak dynamics or inter-annual oscillations in malaria. These are:

1. The inter-annual cycles are driven by climate, and thus should be modeled by external forcing dependent on rainfall, temperature and other climatic covariates. This hypothesis has been investigated on numerous occasions and a number of articles address the impact of El Nino oscillation, and other climatic variables on the dynamics of malaria (Poveda et al. 2001, Laneri et al. 2010, Pasqual et al. 2008).
2. The inter-annual cycles are generated by the intrinsic dynamics of the disease. In this case they presumably should be obtained from autonomous differential equation models. Few studies have been carried out that investigate the possibility that intrinsic reasons are responsible for the inter-annual oscillation and unstable outbreak dynamics of vector-borne diseases. The relatively stable dynamics of even multi-dimensional ODE models, and the relatively recent realization that delay models have the potential to produce oscillations have obstructed more serious studies into the possibility the unstable dynamics may be produced by autonomous deterministic differential equation models. Here, we suggest that, if autonomous, non-stochastic differential equation models have the potential to produce the complex dynamics of vector-borne diseases in nature, these should be differential-delay models.

3. The inter-annual cycles are a result of the joint action of climatic and internal mechanisms. In this case, the baseline autonomous differential equation model on which the stochastic and/or externally forced version is built, should also be able to produce oscillations itself. Hence, this baseline model should be a differential-delay model, rather than an ODE model.

In a recent article Laneri et al. (2010) compare the three options based on an ODE model with external forcing and stochasticity. The results in that article suggested that "the nonlinear dynamics of the disease itself plays a role at the seasonal, but not the inter-annual, time scales." The article seems to settle the question in favor of climatic drivers, but that conclusion is reached in the absence of any understanding in the literature regarding what particular *intrinsic* mechanisms could cause such an unstable, oscillatory or chaotic dynamics. Here, we argue that delay-differential equations are a good modeling tool on which investigation of the intrinsic mechanisms can be built.

4 Discussion

Vector-borne diseases are stable in many regions; however, a closer look reveals that there is diversity in how these diseases manifest in different areas. The mechanisms producing this diversity in disease dynamics are still not well understood. Seasonality in weather is a reasonable mechanism for intra-annual fluctuations in vector-borne disease prevalence because of the dependence of arthropod abundance on rainfall and temperature. However, it is unlikely that climate alone can explain inter-annual oscillations like those observed in Haiti (Fig. 2.2), particularly when the period of these oscillations appears predictable. Because delay-differential equation models are capable of producing inter-annual oscillations, this class of deterministic models appears to be an appropriate choice for exploring the mechanisms behind these less intuitive patterns in disease prevalence. Such exploration could lead to different insights: either intrinsic aspects of vector-borne diseases can cause inter-annual oscillations, seasonality and intrinsic mechanisms may work together to produce inter-annual oscillations, or perhaps neither of these hypotheses is supported and further research is required to find other possible causes of these unstable disease patterns. Regardless of the outcome, it is likely that studying delay-differential equation models for vector-borne disease will contribute to our understanding of unstable transmission, particularly if these models are confronted with data.

Many of the vector-borne diseases have a more complex biology than the models included in this chapter. For instance, individuals infected with *Plasmodium vivax* malaria who have been treated and have recovered from clinical symptoms may relapse (Adak et al. 1998). Furthermore, a malaria infected individual may become bitten by an infectious mosquito and become super-infected with a different strain – a scenario modeled by the concept of multiplicity of infection (Smith and Hay 2009). One individual can become infected by more than one *Plasmodium* species (co-infection). All these scenarios have been captured by ordinary differential equation models (Chiyaka et al. 2010). These ordinary differential equation models with

superinfection, co-infection and relapse can be recast to incorporate delays in the same way discussed in this chapter, although if the different strains have different delay times, it may not be possible to eliminate the dynamic equation for the vector. Still, the resulting delay-differential equations will exhibit competition and coexistence of strains in the context of oscillatory behavior and chaos.

Ruan et al. (2009) study of malaria transmission using a delayed Ross-Macdonald model provided the insight that increasing the duration of either the intrinsic or extrinsic incubation periods would result in reducing the basic reproduction number. This finding has important implications for the future of malaria and malaria control. Climate change, for example, could result in prolonging the extrinsic incubation period in some regions, potentially changing the distribution of malaria, or further increasing malaria prevalence in already endemic countries. More optimistically, it also suggests that there is an opportunity for a different approach to malaria control. The current control measures include larvicides, insecticides, bed nets, and treatment. However, a less traditional approach, such as the use of drugs that prolong incubation periods, may also be an effective means of control.

Another concern that arises from our current knowledge about delay-differential equation models for vector-borne diseases, such as the possibility of Hopf bifurcation, is that changes in the incubation periods may alter the dynamics of the disease, causing a stable transmission region to become unstable, or vice versa. Consequently, understanding if and when these transitions are likely to occur may be very important in determining the effects of climate change, or intervention strategies that prolong incubation periods. Ruan et al. (2009) also suggest that long incubation periods may play an important role in "nonlocal" disease transmission since longer incubation periods means that humans and mosquitoes are more likely to travel long distances prior to becoming infectious or symptomatic. Thus, delays in vector-borne diseases may play a critical role in understanding the spatial spread of these diseases in addition to understanding unstable transmission. The combination of delays and human migration also could potentially contribute to epidemic patterns of transmission.

The indirect transmission between vector and host, the vector's and pathogen's climate-dependent survival, and the relationship between the pathogen's extrinsic incubation period and temperature contribute to the complexity of vector-borne diseases, challenging our understanding of their dynamic and varied behavior in different regions around the world. Stochastic events such as natural disasters or human migrations further complicate and cloud the picture. Understanding the mechanisms producing unstable transmission patterns in order to improve current control efforts seems like a daunting task. However, history has demonstrated the utility of developing mathematical models to understand complicated phenomena such as disease transmission. Consequently, we should feel encouraged that pursuing the study of delay-differential equations in epidemiology may provide similar insight into the mechanisms driving vector-borne disease dynamics in unstable transmission settings. A better understanding of unstable transmission will then allow public health officials to develop intervention strategies more appropriate for these epidemic-prone regions, alleviating the burden on health facilities during outbreaks and mitigating the risk of high morbidity and mortality within a population.

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WHO, Malaria surveillance indicators. http://ais.paho.org/phip/viz/malaria_surv_indicators_popup.asp
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Chapter 3
West Nile Virus: 12 Years in North America

Eleanor Deardorff and Gregory D. Ebel

1 Current Epidemiology

1.1 Global Patterns

West Nile virus (WNV) is a mosquito-borne virus that causes disease in wild birds, horses, and humans. It is one of the best-known members of the flavivirus genus in the virus family *Flaviviridae*. There are approximately 70 members of the genus Flavivirus, and about 40 are known to cause human disease. Most of these are transmitted by the bite of an arthropod such as a mosquito or a tick (Fig. 3.1). Eighty percent of human WNV infections are subclinical or asymptomatic. West Nile fever is the most common disease manifestation and consists of a generalized febrile illness. Malaise, fatigue, headache, nausea, vomiting, diarrhea, and confusion are the most common symptoms. Approximately 30% of symptomatic infections involve neuroinvasive disease that can be fatal or result in long-term sequelae such as persistent movement disorders and personality changes (Sejvar et al. 2003; Murray et al. 2007). A recent report of long-term persistence of WNV RNA in human urine coupled with previous data on the presence of IgM several months after acute WNV disease raises the possibility that several of the long-term sequelae may be due to persistent WNV infection in a subset of patients (Murray et al. 2010). The immunocompromised and elderly are disproportionately affected by WNV infection and are much more likely to develop severe disease than healthy adults under the age of 70. WNV therefore produces a broad spectrum of clinical disease in human beings. The mechanisms that lead to these various manifestations are only partially understood.

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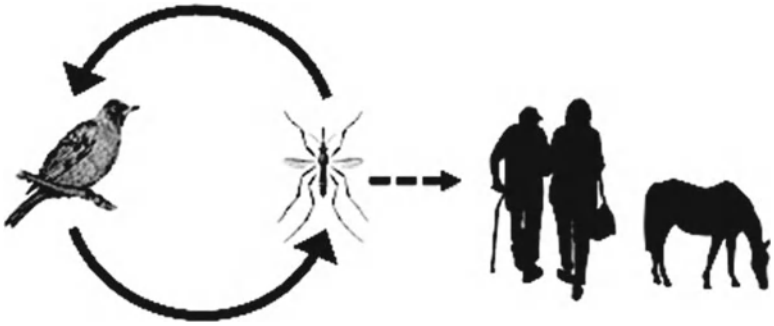

Fig. 3.1 West Nile virus natural transmission cycle. The virus circulates between competent ornithophilic mosquito vectors and avian hosts. Occasional transmission to dead-end hosts such as horses and humans is considered incidental

The original isolation of WNV occurred in Uganda in 1937, and it has been considered endemic in North Africa since the 1950s (Smithburn et al. 1940; Taylor et al. 1956). The largest known epidemic of human West Nile fever occurred in 1974 in South Africa and involved approximately 10,000 cases (Komar 2003). For several decades after its discovery epidemics were reported in Africa, Eurasia, the Middle East and Australia, and little neurological involvement was seen (Hayes 2001). In the last half of the twentieth century major epidemics occurred more frequently and with more severe disease in Egypt (1950), Israel (1951 and 1998–2000), France (1962), Algeria (1994), Romania and Morocco (1996), Tunisia (1997) Italy (1998), and Russia (1999) (L'vov et al. 2004; Murgue et al. 2001). In 2007 the first incidence of human WNV disease in Spain was reported (Kaptoul et al. 2007) and shortly thereafter the first incidence of human neuroinvasive disease in Italy occurred (Rossini et al. 2008). The latter report occurred coincidently with a report of equine disease in the same area.

WNV was first associated with equine disease in the early 1960s (Murgue et al. 2001; Schmidt and Elmansoury 1963). The first evidence that wild birds were involved in transmission cycles came in the early 1950s; however, large-scale die-offs due to natural WNV infection of wild birds were not seen until after 1999 as WNV spread explosively across North America when crow populations were particularly affected (Work et al. 1955; CDC 2000). The introduction of WNV to the New World occurred by way of New York City in 1999 where the first isolate came from a dead American crow (*Corvus brachyrhynchos*) (Lanciotti et al. 1999). Presently WNV has spread to Canada (Canada PHAo 2010), Mexico (Estrada-Franco et al. 2003), the Caribbean (Komar and Clark 2006), and continues to spread throughout Central and South America (Morales et al. 2006; Gubler 2007). Between 2002 and 2009 Canada reported 4,555 cases of clinical disease (Epp et al. 2010). In an interesting contrast, the official number of cases of human WNV infection in Mexico is just seven, despite evidence of widespread continued circulation (Rodríguez et al. 2010). WNV is thus an extraordinarily widely distributed flavivirus that causes a wide spectrum of disease in vertebrates.

WNV has clearly successfully adapted to maximize its potential to perpetuate in a wide array of environments. This has led to intense interest in the evolutionary biology of WNV, and how host–virus interactions shape virus population biology. While the first studies of WNV after its introduction into North America found minimal evidence for adaptive evolution and population variation (Anderson et al. 2001; Beasley et al. 2003; Ebel et al. 2001), subsequent studies comparing the NY99 and WN02 genotypes demonstrated that the dominance of the WN02 genotype is mediated by an approximately 2-day reduction in the amount of time required between mosquito feeding on an infectious host and the appearance of virus in the mosquito salivary secretions (i.e., the extrinsic incubation period, or EIP) (Ebel et al. 2004). Studies of the mechanistic basis that underpins the adaptive potential of WNV have shown that WNV forms genetically heterogeneous populations within each infected individual (Jerzak et al. 2005), and that mosquitoes drive virus diversification through the action of RNAi (Brackney et al. 2009). Further, a high level of population variability contributes to virus fitness by providing a pool of genomic variants that are able to escape RNAi through a mechanism akin to negative, frequency-dependent selection (Fitzpatrick et al. 2010). Moreover, a model of WNV population biology is emerging wherein mosquito infection provides the virus population with adaptive plasticity and infection of vertebrates ensures high virus fitness by exerting strong purifying selection on the variants that arise in mosquitoes (Deardorff et al. 2011).

1.2 United States Public Health Impact

The introduction of WNV into the USA spurred a massive public health response. In the year 2000, the Centers for Disease Control and Prevention (CDC) along with state and local agencies implemented the surveillance system ArboNET for data collection pertaining to WNV infection of humans, mosquitoes, birds, horses, and other animals. It has been determined that the magnitude of bird deaths in a given year can be used to predict the magnitude of human disease later in the year (Komar 2003). Interestingly, it has also been shown that *Culex pipiens* mosquitoes shift their feeding preference from birds to humans in the fall as robins migrate away (Kilpatrick et al. 2006). This sevenfold increase in human feeding is highly correlated with the peak season of disease incidence, as 85% of human cases occur in late summer. These findings supported seminal early studies on *C. pipiens* feeding and reproductive behavior and suggested that mosquito behavior and biology is likely a proximal determinant of public heath risk due to WNV (Spielman 1964, 1967).

WNV is currently the leading cause of arboviral disease in the USA. The WNV strain that was originally introduced to the USA has become known as NY99 (Lanciotti et al. 1999). This strain was quickly displaced by a variant termed WN02, which first appeared in 2001 and become completely dominant by 2004 (Fig. 3.2) (Ebel et al. 2004; Davis et al. 2005; Snapinn et al. 2007). The dominant North American WNV strains have adapted to local mosquito populations and more

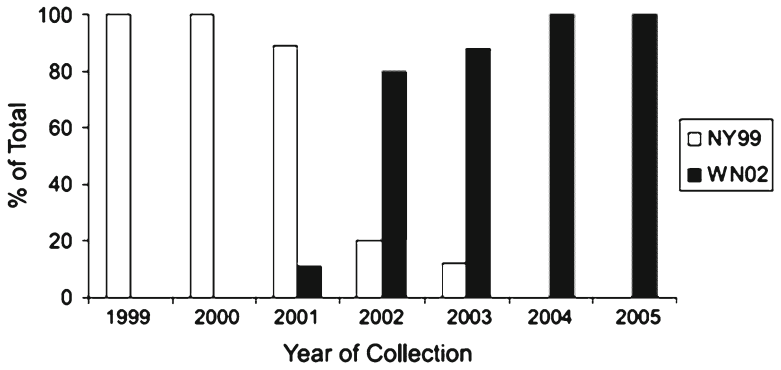

Fig. 3.2 By 2004 the WN02 North American adapted WNV strains had completely displaced the introduced NY99 WNV strains (Snapinn et al. 2007)

efficiently infect North American *Culex tarsalis* and *C. pipiens*, two main vectors of WNV in the USA (Ebel et al. 2004; Moudy et al. 2007). In 2009 the incidence of WNV neuroinvasive disease was 0.13 per 100,000 population, which is a slight decrease compared to 2008 (0.23 per 100,000 population) and a marked decrease from the peak incidence of WNV neuroinvasive disease, which occurred in 2002, of 1.02 per 100,000 population (CDC 2009; Lindsey et al. 2000). A sharp increase in WNV neuroinvasive disease in 2002 and subsequent years compared to 1999–2001 is strongly correlated with the displacement of the NY99 strain by the WNV02 strains. Virus evolutionary and ecological dynamics have thus clearly influenced public health in the USA.

WNV also has influenced blood safety practices in the USA. During the 2002 transmission season the USA experienced the largest WNV epidemic on record with 3,389 cases of human infection and 199 human deaths (Komar 2003). Some of these were the result of transmission though the distribution and use of contaminated human blood products. Following 23 occurrences in 2002 of WNV infection by blood transfusion or organ transplant, nationwide screening was implemented in the summer of 2003 (FDA 2009). There are now two nucleic acid test kits licensed by the FDA for providers of blood products to screen donations (FDA 2009). Blood, blood products, and organs are routinely tested for WNV infection; however, screening is not standardized and transmission by these routes still occasionally occurs (CDC 2008; Diamond 2009).

1.3 Mathematical Modeling

The study of how a pathogen, its host, and its vector interact spatially and temporally with each other and the environment is called landscape epidemiology (Fig. 3.3). The use of geographic information systems (GIS) in conjunction with remote sensing

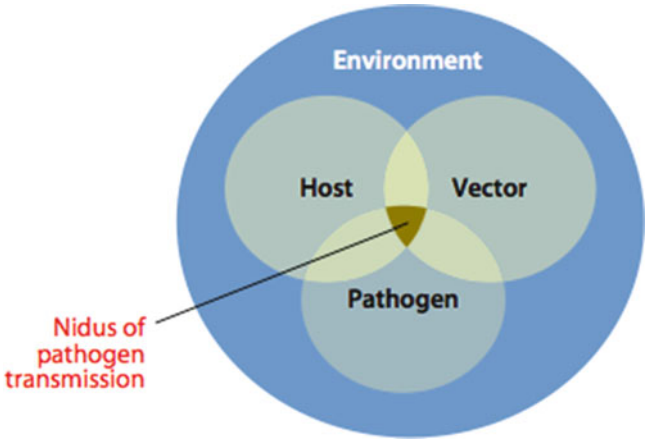

Fig. 3.3 Concept map showing how pathogen, host, and vector populations intersect within a permissive environment to allow pathogen transmission (Reisen 2010). Reprinted with permission from Annual Review of Entomology

and modern computer technology is transforming the field of landscape epidemiology. These technologies are powerful tools for modeling disease dynamics and predicting future trends. Static and dynamic variables are used to calculate expected increases and decreases in mosquito infection rates, incidence in human beings, areas and/or times of greatest risk, etc. (Keeling and Rohani 2008). Output from these models could be used to inform public health and vector control strategies (Fig. 3.4).

Numerous studies have created multivariate maps in a variety of regions of the USA in order to determine which environmental, ecological, or economic factors best predict WNV exposure risk. For example, human risk maps were derived for the state of Mississippi using landscape and climatic data and were validated with existing human case data (Cooke et al. 2006). The results suggested a spatial correlation between bird fatalities and human risk but surprisingly found no clear distinction between urban and rural areas. In a similar study performed in Colorado, a multivariate regression modeling approach was used to create two models for WNV exposure risk—one for the Eastern plains landscape and the other for the Western mountainous region (Winters et al. 2008). The results indicated river-proximity, human population density and irrigated agricultural land cover were predictive factors for higher WNV risk in the Eastern region. The two models were robust and noninterchangeable, emphasizing the need for precision with respect to data and parameter selection when designing a model.

Remote sensing technology has improved our ability to gather ecological and geographic data for GIS-based landscape epidemiology studies with a fraction of the time and effort previously required. Using a case–control approach with ecological, geographic, and socioeconomic data, Rochlin et al. created a human risk map in Suffolk County NY (Rochlin et al. 2011). A logistic regression model was used for GIS analysis of data from 2000 to 2004 and was found to accurately predict 89% of human WNV activity for the subsequent years (2005–2010).

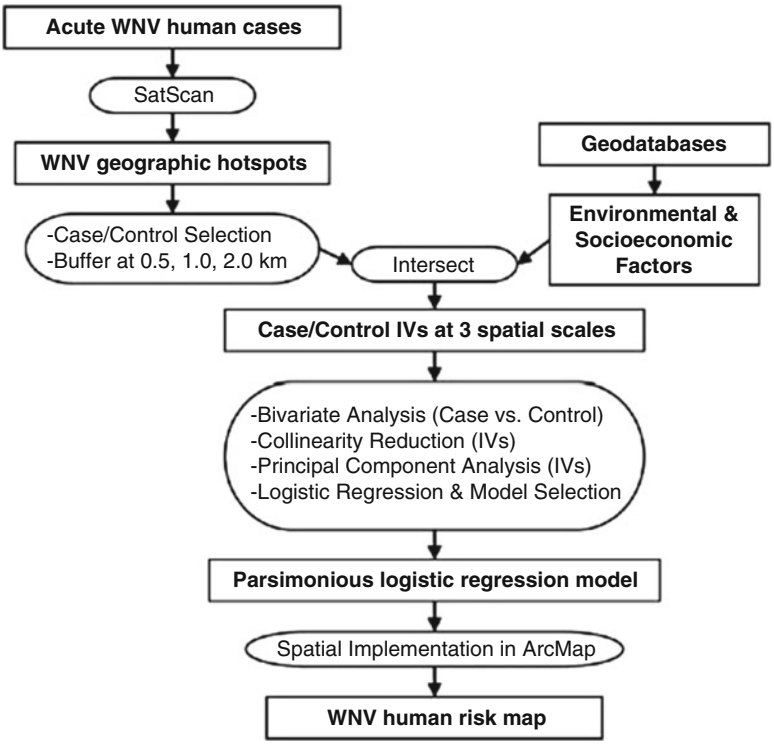

Fig. 3.4 Procedure for logistic regression model construction and West Nile virus (WNV) human risk map development. IVs-independent variables (Rochlin et al. 2011)

Another tool being used in WNV human risk prediction mapping is called maximum entropy species distribution modeling, or Maxent. This method is based on a probabilistic framework and makes the assumption that the incomplete empirical probability distribution (occurrence data) can be accurately approximated with a maximum entropy probability distribution (Phillips et al. 2006). Several recent studies have employed the Maxent technique to develop human WNV risk maps. Larson et al. report a simple method for using ecological niche modeling to create probability distribution maps of human WNV incidence in Iowa (Larson et al. 2010). In a comparison of two approaches, Maxent ecological niche models were found to be more accurate than genetic algorithm for rule-set prediction (GARP) models for WNV vector mosquitoes. When the Maxent modeled niches were averaged across mosquito species an informative static human risk map was created. The Maxent approach was also used with a combination of economic census data and remotely sensed ecological factors to predict variation in WNV vector infection and human disease in Orange County CA (Harrigan et al. 2010). This study found a negative correlation between WNV prevalence and economic status with per capita income and density of neglected swimming pools emerging as the most accurate predicting variables for vector and human infection from 2005 to 2009.

Modeling can also address questions of wildlife ecology and how it relates to WNV transmission and associated human disease risk. Using data collected by the North America Breeding Bird Survey (BBS) and a remotely sensed land-use classification the impact of anthropogenic change on WNV ecology was examined (LaDeau et al. 2010). Increasing urban land cover and the associated increasing winter temperatures were correlated with declines in crow populations beyond those attributed to WNV infection alone. Ecological models have also been created to help predict risk in anticipation of the arrival of WNV and also to compare and contrast the effects of related viruses with different ecologies (Tachiiri et al. 2006; Lord and Day 2003). Modern modeling approaches when combined with empirical data can help formalize our understanding of the dynamics of WNV transmission.

2 Basic Science

2.1 Clinical Pathology

Although the majority of infections are asymptomatic and the majority of disease is mild, WNV remains a significant cause of morbidity and mortality because so many individuals are infected. Approximately 1 in 150 infections result in severe life-threatening illness with a much greater incidence of neuroinvasive disease and death in those over 50 years old (Diamond 2009). Neurologic disease is most often characterized as encephalitis (inflammation of the brain), meningitis (inflammation of the meninges), or mengioencephalitis (inflammation of the brain and meninges). Clinical symptoms of neurologic involvement are varied and inconsistent but include slurred speech, tremors, shortness of breath, abdominal pain, focal sensory changes, pharyngitis, conjunctivitis, seizures, and lymphadenopathy. Several severe manifestations have been reported that were not associated with WNV infection until after the virus invaded North America. These include poliomyelitis-like acute flaccid paralysis, profound muscle weakness, Guillain–Barré syndrome, and ocular abnormalities (Leis et al. 2002; Sejvar et al. 2005).

According to the CDC, the clinical criteria for diagnosis of neuroinvasive disease require the presence of fever and at least one of the following signs: altered mental status (disorientation, stupor, coma), other acute sign of neurologic dysfunction (paralysis, nerve palsies, sensory deficits, convulsions, abnormal movements or reflexes), or increased white blood cells in the cerebrospinal fluid associated with headache and/or a stiff neck (Lindsey et al. 2000). There were 11,822 cases of neuroinvasive disease reported in the USA between 1999 and 2008 (Lindsey et al. 2000). The median age of these was 57 years (range: 1 month–99 years), 58% were males, 88% were Caucasian, and 1,045 cases were fatal.

In recent years it has become clear that recovery from acute WNV infection may be prolonged and accompanied by persistent symptoms, including weakness, persistent movement disorders, neurologic impairment, and cognitive difficulties (Sejvar et al. 2008; Sejvar 2007). Data from studies of WNV in mice, birds, and hamsters have suggested that the virus may establish a persistent infection in

particular tissues (Appler et al. 2010; Nemeth et al. 2009; Tonry et al. 2005). Most notably, the renal tropism of WNV in birds (which has made bird kidneys a preferred tissue for WNV surveillance programs) seems to be recapitulated in mammals (Tonry et al. 2005). Importantly, WNV RNA has been demonstrated in the urine of human beings several years after "recovery" from acute infection (Murray et al. 2010). Thus like other flaviviruses, WNV may establish persistent infection in a subset of individuals, presenting an additional and generally unrecognized health burden in the USA (Ogawa et al. 1973).

2.2 Risk Factors

Several epidemiological studies have addressed factors associated with the outcome of WNV infection. More severe disease (i.e., West Nile encephalitis and/or death) is associated with advanced age and age-associated comorbidities such as diabetes and history of stroke (Bode et al. 2006). Reduced immune function, which is generally associated with advanced age, is also a major risk factor for WNV infection and immunocompromised individuals are more likely to develop disease. One study reported that up to 60% of immunosuppressed patients receiving a solid organ transplant from a WNV infected donor will develop WNV neuroinvasive disease (CDC 2008).

Recently two genetic risk factors for human WNV disease have been described. The human chemokine receptor CCR5 is thought to be partially protective against symptomatic WNV infection. A cohort of laboratory-confirmed, symptomatic, WNV infected patients was found to be homozygous for a defective form of the gene four times more frequently than in a healthy control cohort (Glass et al. 2006). Variation in another gene, the oligoadenylate synthetase gene, is a risk factor for initial establishment of WNV infection but was not correlated with disease severity (Lim et al. 2009).

There are also several ecological risk factors that are considered important to the amount of WNV exposure and consequently the number of human cases. Rainfall and temperature greatly affect mosquito populations and have been correlated with the amount of WNV infection of *Culex* mosquitoes (Ruiz et al. 2010). There is also variation between WNV strains and mosquito vectors in their ability to effectively transmit WNV to humans. For example, the highest incidence of neuroinvasive disease in the USA occurs in the West Central and Mountain regions, which corresponds geographically with the range of *C. tarsalis*, a highly efficient WNV vector (Lindsey et al. 2000).

2.3 Immune Response

The immunological basis for the increased susceptibility of the elderly and immunocompromised for WNV infection is not yet known. Research toward elucidating this and toward basic understanding of the mechanisms underlying disease relies

largely on laboratory animal models. In animals both the humoral and cellular immune responses have been shown to be important in protection from WNV disease (Diamond et al. 2003a). Mice that are unable to produce secreted IgM showed increased vulnerability to severe signs of WNV infection but were rescued by the passive transfer of IgM, indicating the importance of a neutralizing antibody response in the control of infection (Diamond et al. 2003b).

Recently it has been shown that the level of peripheral regulatory T-cells (CD3+ CD4+ CD25hi CD152+ CD127− T-cells) is inversely proportional to the severity of disease. Animals and humans with the highest levels of Tregs had asymptomatic infections. Animals and humans with clinical signs of infection had intermediate Treg levels, and Treg-deficient animals developed lethal infection at a higher frequency than controls (Lanteri et al. 2009). CD8+ T-cells are thought to be important to clearing virus from the periphery and to preventing viral persistence (Shrestha and Diamond 2004), and CD4+ T-cells have been found to be essential for clearance of WNV from the central nervous system (Sitati and Diamond 2006). Therefore, protection from severe WNV disease seems to be a balance between robust activation of innate and acquired immune responses, and appropriate control of these responses.

3 Disease Control

3.1 Therapeutics

The current treatment for WNV infection consists largely of supportive therapy and much research is devoted to discovering antiviral treatment strategies. Nucleoside/nucleotide analogs are a class of molecules that are often investigated for their antiviral properties. These compete with endogenous nucleosides/nucleotides and disrupt viral genome replication. Ribavirin is a popular antiviral drug that acts on this principle. It is a guanosine analog that has been shown to be an effective antiviral agent in the treatment of several important viral diseases including influenza and hepatitis C viruses (Davis et al. 1998; Rowe et al. 2010; Eriksson et al. 1977). Although this drug was shown to be efficacious against WNV infection in cell culture, experimental infection of hamsters with WNV showed increased mortality after ribavirin treatment (Day et al. 2005; Morrey et al. 2004). Additionally, ribavirin therapy was retrospectively found to be statistically correlated with fatal outcome during a human WNV epidemic in Israel (Chowers et al. 2001).

Another avenue of research into therapeutics is the administration of exogenous antibodies to prevent or mitigate disease. Passive transfer of human gamma globulin prior to challenge completely protected wild-type mice from lethal WNV infection (Engle and Diamond 2003). Postexposure treatment provided partial protection even when administered 5 days after exposure when the virus had already reached the central nervous system. In a subsequent study, monoclonal antibodies against WNV were produced in plants and were shown to be as protective as mammalian-derived antibodies and able to be rapidly produced in large quantities (Lai et al. 2010).

High affinity function-blocking human antibody fragments specific to the active site of the viral proteinase have been identified through recombinant antibody technology (Shiryaev et al. 2010). This approach uses protein sequence and structure to engineer specific inhibitory antibody fragments and allows for genetic manipulation and modification making it broadly applicable to a variety of systems beyond WNV.

A relatively new branch of therapeutics research that is receiving much attention involves various forms of RNA. One of these processes, RNA interference, occurs in nearly all orders of life and results in RNA molecules within the cell being degraded in a sequence-specific manner. Cellular double-stranded small interfering RNA (siRNA) and cytoplasmic ribonucleases work to target and degrade single-stranded RNA molecules such as the WNV genome. The administration of siRNA is protective to mice if administered before WNV challenge or within 6 h postinfection, but is not therapeutic when administered 24 h postchallenge (Bai et al. 2005; Kumar et al. 2006). Sequence-specificity of viral RNAs can also be exploited in the development of oligomers that hybridize with and thus block single-stranded viral RNA. This antisense technology has proven effective against WNV in a variety of systems and has resulted in at least one candidate entering clinical human trials (Diamond 2009).

There are numerous other molecules that are being examined for their ability to prevent or diminish the effects of WNV and other flavivirus infection. Interferon-alpha, an important component of the innate immune system, appears to be involved in controlling WNV infection. Treatment with exogenous interferon has been shown to prevent or reduce flavivirus infection in mice and reduced complications in human infections (Diamond 2009). There is evidence that matrix metalloproteinase inhibitors aid in ameliorating the disruption of the blood–brain barrier that is commonly seen after infection with WNV (Verma et al. 2010). These thus represent a class of molecules worthy of further therapeutic consideration.

3.2 Vaccines

Because of the lack of effective therapies, much research has focused on vaccine development. Currently, there are several WNV vaccines licensed for veterinary use, but none have been approved for use in humans (Table 3.1) (Kramer et al. 2008). In order for a vaccine to be approved for humans it must undergo a long series of tests and trials to demonstrate safety, efficacy, and practical utility. A candidate vaccine must be proven in two small animal models and a nonhuman primate model before being considered for human clinical trials. Vaccine preparations can use viral protein subunits or nucleic acids, killed or inactivated virus or live-attenuated virus as immunogens. Each of these approaches carries concerns with respect to safety and stability, particularly the live virus vaccines. A vaccine candidate must be evaluated for potential transmission by arthropod vectors, reversion to virulence, recombination with a second virus, or contamination with known or unknown agents from the animal system in which the vaccine was produced.

Table 3.1 WNV vaccines that are licensed for veterinary use or in clinical trial

Product name	Company and/or institute	Vaccine type	Status
Innovator®	Fort Dodge Animal Health	Killed virus	L
Recombitek®	Merial	Recombinant canarypox virus	L
PreveNile™	Intervet	Chimeric virus (WNV/YFV)	L
NA	Kimron Veterinary Institute/Crucell	Killed virus	L
NA	CDC/Fort Dodge Animal Health	Recombinant DNA plasmid	L
Chimeravax™-West Nile	Acambis	Chimeric virus (WNV/YFV)	CT-II
VRC-WNVDNA020-00-VP	NIAID/NIH	Recombinant DNA plasmid	CT-I
WN/DEN4-3′ delta30	NIAID/NIH	Chimeric virus (WNV/DEN4)	CT-I
NA	Crucell	Killed virus	CT-I

NA information not available, *L* licensed for veterinary use, *YFV* yellow fever virus, *CT-II* clinical trial, phase II, *CT-I* clinical trial, phase I, *DEN4* dengue-4 virus (from Kramer et al. 2008). Reprinted with permission from Annual Review of Entomology

One approach under development for vaccine production that mitigates these concerns is the use of viral subunits that are incapable of propagating, reverting to virulence, or recombining. These subunit vaccines usually comprise the entire or partial prM and E structural proteins of the WNV virion, and are combined with an adjuvant to increase potency. A subunit vaccine comprised of domain III of the E protein conjugated to virus-like particles from a bacteriophage showed good protection against lethal challenge in mice (Spohn et al. 2010). Another subunit vaccine consisting of the E protein domain III and adjuvanted with the P28 region of the complement protein C3d successfully protected mice from WNV infection (Dunn et al. 2010). Aluminum hydroxide has also been used to adjuvant a WNV subunit vaccine with success at preventing infection and boosting preexisting antibody titers in mice, hamsters, and foals (Bonafé et al. 2009). Nanolipoproteins as the adjuvant represent a versatile antigen presentation and delivery platform for histidine-tagged viral protein antigens. This approach has shown promise when employed toward a WNV vaccine; however, toxicology, biodistribution, and stability studies are still in progress (Fischer et al. 2010).

A second method of potentially circumventing some of the safety concerns is to remove the need for vaccine preparation in vivo. Synthetic biology that does not rely on animal isolates is a promising new technique in live vaccine research (Cello et al. 2002). De novo chemical cDNA synthesis of a WNV vaccine produced an inactivated virus that was comparable to the wild-type parent in terms of virus growth as well as virulence and neutralizing antibody production in mice and no undesired mutations were detected in the rescued WNV genome (Orlinger et al. 2010). This technology may eliminate some of the genetic instability associated with live animal propagation of viruses for vaccines and also eliminates the concerns for contamination with other known or unknown agents.

A popular approach in live-attenuated vaccine development is genetic manipulation to create chimeric viruses in which genes from a pathogenic virus are substituted into the genetic backbone of a related nonpathogenic virus. A chimeric WNV vaccine has been developed using ChimeriVax™ technology that replaced the prM and E genes of the 17-D yellow Fever virus vaccine strain with those from heterologous WNV (Guy et al. 2010). One preparation of this live-attenuated vaccine has been approved and is commercially available as a horse vaccine. A modified version, ChimeriVax-WN02, which includes three putatively redundant attenuating point-mutations in the E gene was found to be safe and immunogenic in humans during phase I clinical trials and is currently undergoing phase II trials (Guy et al. 2010; Arroyo et al. 2004; Monath et al. 2006).

Another interesting avenue of live-attenuated vaccine research is single-cycle virus technology. A portion of the virus's genome is deleted making it able to infect but unable to propagate, thus producing viral antigens without perpetuating infection. An attenuated single-cycle virus vaccine RepliVAX-WN has recently been reported as safe and efficacious in a nonhuman primate model and may be another promising candidate for a human vaccine (Widman et al. 2010). Several other live vaccines that contain various attenuating deletions or mutations have been developed and have so far been shown to be efficacious in laboratory animal models (Schlick et al. 2010; Whiteman et al. 2010).

Vaccination of wildlife to disrupt the natural transmission cycle is another area of important vaccine research. A recombinant plasmid DNA vaccine that expresses the prM and E proteins has been licensed for use in horses (Davis et al. 2001) and is being investigated for use in wild birds. It was recently evaluated in American Robins (*Turdus migratorius*), a key species in the natural life cycle of WNV in North America, and was found to reduce the average viremia to a level not likely to infect feeding mosquitoes (Kilpatrick et al. 2010). This was an injected vaccine preparation however and until it can be orally administered the application to wild robin populations is limited. Injection of this vaccine was also successful where oral administration failed in fish crows (*Corvus ossifragus*) (Turell et al. 2003) American crows (*C. brachyrhynchos*) (Bunning et al. 2007) and the endangered California condor (*Gymnogyps californianus*) (Chang et al. 2007).

3.3 Vector Control

Since therapeutics and vaccines are not yet available, current preventive measures are focused on minimizing exposure to the mosquitoes that transmit WNV. These measures include reducing larval habitat and larval viability as well as reducing adult viability and vector competence. Although mosquito control is the most accessible means of mitigating WNV infection, it is inconsistent or altogether absent in many areas where WNV is endemic.

Monitoring mosquito vector populations and performing surveillance for WNV-infected mosquitoes is a reliable and accurate indicator of WNV circulation.

In 2005 an outbreak of WNV occurred in Sacramento County, California. Emergency aerial spray of pyrethrins was employed to control adult mosquitoes. The results were a 58% decrease in abundance of WNV vector mosquitoes, a reduction in the infection rates of captured mosquitoes and a reduction in the number of human cases (Elnaiem et al. 2008; Carney et al. 2008).

Although pesticides are effective, there are concerns regarding potential toxicity in the environment and also the eventual development of resistance in wild mosquito populations. In light of this, biopesticide research is receiving renewed interest. There are several bacteria, viruses, and fungi that infect and kill mosquitoes that may be developed as specific and nontoxic alternatives to chemical pesticides (Kramer et al. 2008).

In addition to altering the ecology of the vectors, human behavior can be altered to reduce exposure to WNV transmitting mosquitoes. Recommended measures to protect humans from WNV exposure include the use of protective clothing when out of doors, the use of insect repellent on skin and clothing, the avoidance of outdoor activities from dusk till dawn, the elimination or tight covering of standing water sources near homes, the installation and maintenance of window and door screens, and the vaccination of all horses.

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Chapter 4
Leishmaniasis: An Update on a Neglected Tropical Disease

Amber Read, Ivy Hurwitz, and Ravi Durvasula

1 Introduction

Leishmaniasis is a devastating and significantly under-recognized vector-borne disease causing serious global health burden. The disease is caused by *Leishmania*, a protozoan kinetoplastid parasite. Though the disease is most prevalent in certain endemic locales, the disease spans 88 countries in the tropics, subtropics, and Southern Europe (Herwaldt 1999). After disease is acquired through the bite of an infected phlebotomine sand fly, disease can manifest in various forms from cutaneous disease to mucocutaneous involvement to visceral disease in which splenomegaly, cachexia, and anemia are salient features and the disease is virtually fatal if untreated (CDC http://www.cdc.gov/parasites/leishmaniasis/disease.html).

Ninety-percent of cutaneous forms of leishmaniasis are localized to Afghanistan, Algeria, Brazil, Peru, Saudi Arabia, and Syria (Alvar et al. 2006; WHO: http://www.who.int/leishmaniasis/burden/en/; Desjeux 2004a,b). Alternatively, 90% of visceral leishmaniasis cases are found in India, Bangladesh, Nepal, Sudan, Ethiopia, and Brazil (http://www.cdc.gov/parasites/leishmaniasis/epi.html; Desjeux 2004a,b). Of these five countries, India carries the greatest burden of disease with 50% of all cases occurring in India alone. The disease has further regional predilection within India, with 90% of India's cases occurring in Bihar, one of the poorest of Indian states (Singh et al. 2006).

It is estimated that 1–1.5 million new cases occur per year of cutaneous leishmaniasis and 500,000 new cases occur per year of visceral leishmaniasis (Desjeux 2001a,b; Desjeux 2004b). Prior estimates have suggested that 12 million people are infected with leishmaniasis and 350 million people in the world remain at risk of developing leishmaniasis. These numbers, staggering as they are, are likely a gross

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underestimate. Leishmaniasis burden is greatest in developing countries where accurate case reporting is lacking. The disease is classified as one of the most neglected tropical diseases and is second only to malaria in terms of parasitic disease mortality (Alvar et al. 2006). Only 32 countries have case reporting (WHO 2010). In one study in India, disease reporting underestimated disease incidence by more than eight times due in part to lack of active case finding and the majority of patients presenting for care to private institutions where case reporting does not occur (Singh 2006). In addition, a significant number of people do not seek care and remain undiagnosed. Occasionally patients recover without symptomatic onset of disease and are not factored into disease prevalence.

The disease occurs in the "poorest of the poor." Malnutrition worsens the clinical manifestations, poverty creates environmental circumstances which encourage sand fly breeding sites, war and poverty increase migration of unimmune populations to endemic disease regions and migration of infected people into regions previously devoid of disease where local populations have no immunity, establishing new endemic locations (Alvar et al. 2006). Between 1984 and 1994, 100,000 people of a population size of 280,000 died in an epidemic of leishmaniasis initiated by famine and war causing increased malnutrition and migration of an unimmune population into visceral leishmaniasis endemic regions (Alvar et al. 2006). Endemic areas without resources cannot often afford prevention and treatment modalities, worsening the vicious cycle of disease and further propagation of poverty in affected areas (Sarnoff et al. 2010). Following the trend of other vector-borne diseases, as human sprawl continues to encroach upon natural habitats the number of cases is expected to grow (WHO 2002). The number of cases is additionally increasing resulting from HIV coinfection (Desjeux 1999).

Treatment of the disease has been difficult on many levels. Treatment of visceral leishmaniasis is often cost prohibitive. For example, in Sudan, the average yearly wages for a family is approximately $396 (US dollars) and treatment costs approximately $100 per patient, a staggering percentage of the family's yearly livelihood. This figure additionally does not account for lost wages and disability as a result of illness (Alvar et al. 2006; Boelaert et al. 2009; Griekspoor et al. 1999). Though some forms of cutaneous leishmaniasis will regress spontaneously within one year, there are other forms of cutaneous leishmaniasis as well as mucocutaneous leishmaniasis that are difficult to treat in best-case scenarios. In many areas where leishmaniasis is prevalent, the lack of resources precludes treatment with available drugs. In some areas where drugs were used incorrectly as a result of lack of resources and lack of knowledge, drug resistance is becoming prevalent. In India, *Leishmania donovani* resistance is as high as 40% to pentavalent antimony (Sundar et al. 2006a,b,c). Antecedent immunosuppression with HIV and transplantation is increasing relapse rates and the risk of developing active kala-azar in endemic countries is increased by 100–2,320 times because of HIV (http://www.who.int/leishmaniasis/burden/hiv_coinfection/burden_hiv_coinfection/en/index.html). HIV coinfection has now been documented in 35 countries (Roberts and Janovy 2009; Griekspoor et al. 1999).

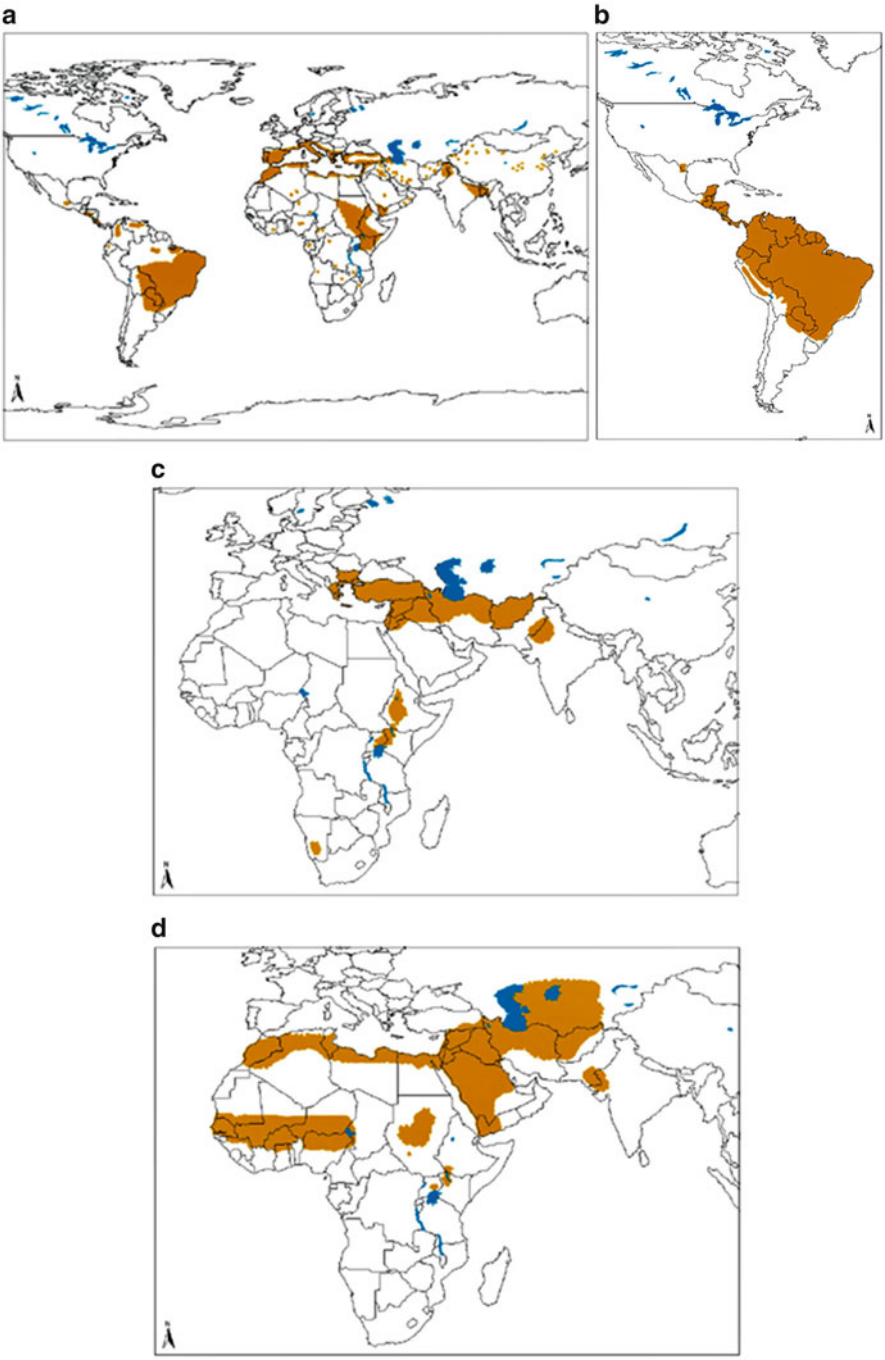

Fig. 4.1 (**a**) Geographical distribution of visceral leishmaniasis in the Old and New world (**b**) Geographical distribution of cutaneous and mucocutaneous leishmaniasis in the New World (**c**) Geographical distribution of Old World cutaneous leishmaniasis due to L. tropica and related species and L. aethiopica (**d**) Geographical distribution of Old World cutaneous leishmaniasis due to L. major WHO/NTD/IDM HIV/AIDS, Tuberculosis and Malaria (HTM) World Health Organization, October 2010 Reprinted with permission of the World Health Organization, http://www.who.int/leishmaniasis/leishmaniasis_maps/en/. Accessed August 15, 2012

Prevention strategies have been a mainstay of leishmaniasis control. Attempts at preventing sand fly bites with bed nets, decreasing sand fly breeding sites, targeting reservoir hosts, and using insecticides to decrease sand fly populations are presently underway. The success of these measures is leaving much to be desired in terms of prevention outcome. Vaccine development continues but there are presently no available vaccines. It has become clear that a multifaceted approach will be necessary to decrease rates of leishmaniasis. Novel prevention strategies are needed to help combat disease.

2 History of Leishmaniasis

Old World cutaneous leishmaniasis has a longstanding documented history. The first descriptions of the "oriental sore" were found on tablets belonging to King Ashurbanipal in the seventh century BC but were likely taken from information first recorded in 1500–2500 BC. In the tenth century, Arab physicians described what is referred to as the "Balkh" sore in Afghanistan (Cox 2002). Later reports showed a similar disease in Baghdad and Jericho. Throughout history, different regions have given different names to the clinical sores characteristic in a particular region. New World cutaneous and mucocutaneous leishmaniasis was well depicted in fifth century AD sculptures and sixteenth century Spanish missionary writings (Cox 2002). Fifteenth and sixteenth century writings describe Andean or valley sickness manifesting as cutaneous sores. Later mucosal disease was again described and referred to as white leprosy (WHO: http://www.who.int/leishmaniasis/history_disease/en/index.html). Paleopathology evidence has confirmed the presence of ancient mucocutaneous leishmanaisis (Fig 4.2) (Costa et al 2009). James Homer Wright is credited with the discovery of the parasite causing cutaneous leishmaniasis, though the parasites were seen initially by David Cunningham in 1885 and Peter Borovsky, a Russian surgeon who published a paper accurately describing the parasite as a protozoan. Borovsky's work went unrecognized as it was written in Russian (Cox 2002).

Visceral leishmaniasis was described much later. There was no documentation of the disease until 1823, despite clear documentation of other diseases causing the same degree of severity, likely indicating that it had not occurred prior to this point (Desowitz 2001). It was noted in 1824 that an outbreak of the disease causing fever and cachexia did not respond to quinine (Cook 2007) in Jessore, Bengal. The disease was called kala-azar or black fever because of the darkening of the skin seen in Indian patients with the disease and was also called Dum Dum fever because of its occurrence in Dum Dum near Calcutta. Outbreaks continued next in Bardwan in 1862 then Asslam in 1869. Initially it was theorized that the significant anemia and splenomegaly was secondary to a combination of hookworm, beriberi, and malaria.

Others doubted this explanation and felt that it was a separate, yet to be discovered disease (Desowitz 2001). Improved transportation systems were put into place and the disease continued to migrate. The disease killed up to 25% of the population in certain areas. Drs. William Boog Leishman and Charles Donovan were credited with the discovery of the *Leishmania* parasites causing kala-azar. Dr. Leishman was initially

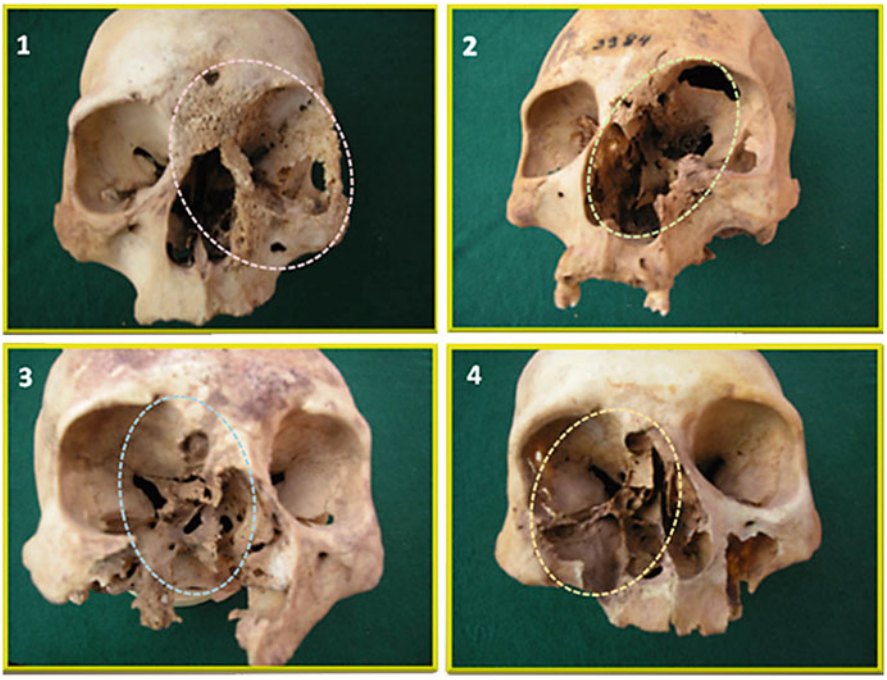

Fig. 4.2 Female skulls from Coyo Oriental cemetery (dating back 500-1000 years), San Pedro de Atacama Northern Chile. Three of the skulls had evidence of Leishmania DNA (Costa et al 2009)

from Glasgow and trained in the Army Medical School. He was sent to India with the Army Medical Service and later returned to Netley. There, he found a parasite in the spleen of a soldier who had died of Dum Dum fever. He initially misclassified the parasite as a trypanosome. He published the results in 1903. At the same time, Dr. Donovan, an Irishman serving in the Indian Medical Service in Dargai then Madras, independently sketched the parasites from the spleen of an ill patient. It was also realized that this was the same parasite isolated from the oriental sore previously. It was not until 1940 that C.S. Swamirath and Henry Edward Shortt successfully proved sand fly transmission of *Leishmania* to people (Desowitz 2001) (Fig. 4.3).

3 *Leishmania* Classification

As alluded to earlier in the chapter, there are a number of forms of disease attributed to *Leishmania* spp. There are approximately 25 species known to cause human disease depending on the classification scheme used (Schönian et al. 2010). Leishmaniasis is often categorized into New World and Old World disease and disease can be anthroponotic when the cycle involves only the sand fly and human populations and

Fig. 4.3 Sir William Boog Leishman (left) and Major Charles Donovan (right). Reprinted with permission from the Wellcome Library, London

zoonotic when the cycle involves humans, sand flies, and mammalian reservoirs, with humans usually becoming an accidental hosts. Each species has certain endemic regions and may cause certain clinical manifestations, though clinical manifestations may vary even with the same species and within the same geographical location. Some of the initial phylogeny categorization was based on location, parasite characteristics, clinical manifestations, and isoenzyme evaluation. Table 4.1 depicts an updated classification scheme using multi-locus enzyme electrophoresis, presently considered the gold standard for classification (Buitrago et al. 2011; Schönian et al. 2010; Correa et al. 2005; Cupolillo et al. 1994). Others argue that *Leishmania* phylogeny should be based on geography, clinical presentation (as treatment response and clinical severity may differ between regions), as well as genetic evaluation.

4 Transmission Cycle of *Leishmania*

Leishmania parasites are transmitted by the bite of an infected female sand fly (Fig. 4.4). The female sand fly requires blood for egg maturation (WHO 2010; Baker et al. 2007). With the blood meal, the female sand fly ingests macrophages containing amastigotes from an infected human or, in cases of zoonotic disease, from an infected mammalian zoonotic reservoir. The acquired macrophages are usually from infected phagocytic cells found in the skin or from amastigotes free and in peripheral blood mononuclear cells in the blood (Singh 2006). The amastigotes migrate to the midgut (*Leishmania*) or the hindgut (*Viannia)* where they transform into flagellated promastigotes and divide. The extracellular promastigotes undergo further transformation, migration, and binding before some turn to metacyclic

Table 4.1 Mammalian *Leishmania* spp

Updated Classification
Section Euleishmania
Subgenus Leishmania *(Old and New World)*
L. donovani complex
*L. donovani** (Old World)
*L. archibaldi**** (Old World)
*L. infantum** (Old World, syn *L. chagasi*)
L. tropica complex (OW)
*L. tropica**
*L. killicki**
*L. aethiopica**
L. major complex (OW)
*L. major**
L. gerbilli
L. arabica
L. turanica
L. mexicana complex (New World)
*L. mexicana**
*L. amazonensis**
L. aristisdesi
*L. venezuelensis**
*L. forattinii**
Other species
L. enriettii
Subgenus Viannia *(New World)*
L. braziliensis complex
*L. braziliensis**
*L. peruviana**
*L. guyanensis** complex
*L. guyanensis**
*L. panamensis**
*L. shawi**
Other *L. Viannia* species
*L. naiffi**
*L. lainsoni**
*L. lindenbergi**
L. utingensis (only from sand fly)
Section Paraleishmania
*L. columbiensis**
*L. equatorensis**
*L. hertigi**
*L. herrei**
*L. deanei**

Reprinted from Trends in Parasitology, Vol 26(10), Schonian et al., Is it time to revise the nomenclature of Leishmania?, page 267, Copyright (2010), with permission from Elsevier

[a] Denotes species that cause human disease

[b] Jamjoom et al. 2004 indicates that *L. archibaldi* is an invalid species

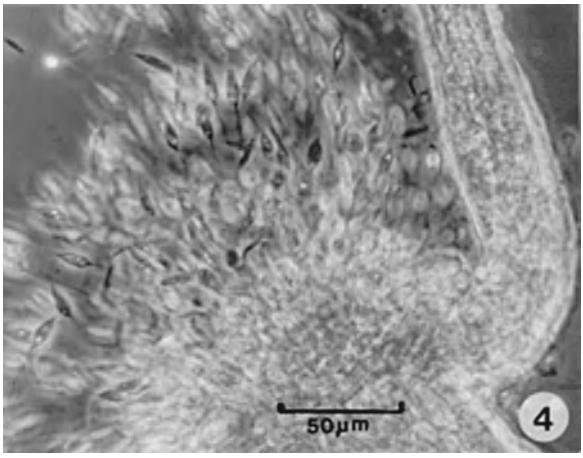

Fig. 4.4 "Ruptured, anterior part of a sand fly gut naturally infected with promastigotes of Leishmania. Large numbers of flagellates are seen exuding from the broken end of the intestine. Fresh preparation, phase-contrast microscopy." Reprinted from Lainson (1997) On Leishmania enriettii and other enigmatic Leishmania species of the neotropics, Mem Inst Oswaldo Cruz 92(3): 382, with permission from Memórias do Instituto Oswaldo Cruz

promastigotes, the infective form. The metacyclic promastigotes travel to the salivary glands where they ready for transmission to the next host. Interestingly, female sand flies, unlike mosquitoes, are not only hemophagenous. They additionally require sugar meals. During initial lab experiments designed to prove that the sand fly transmitted *Leishmania*, it was not until the sand flies were given sugar meals after ingestion of parasites that transmission from the salivary glands was actuated (Desowitz 2001). For many of the parasites and sand flies, maturation occurs within 1–2 weeks and often development can be completed during one blood meal cycle, though some sand flies will continue to take further blood meals during this time period. There are several effects that the parasite has upon the sand fly to make infection successful including interference with digestive enzymes, secretion of peptides that slow peristalsis, damage of the stomodeal valve to assist with transmission and they additionally affect the sand fly behavior, increasing the number of sand fly blood meal feedings (Ramalho-Ortigao et al. 2010; Rogers and Bates 2007). Upon the next sand fly blood meal, the promastigotes are transmitted to the human host and are phagocytosed by macrophages and other phagocytic cells. During this phase of the life cycle, the *Leishmania* parasites transform into amastigotes and live intracellularly. They reproduce by binary fission. Once the cell is full of amastigotes, the cell bursts and amastigotes are released to infect other cells. The parasite is able to avoid macrophage killing by a number of elaborate mechanisms ranging from avoidance of triggering the respiratory burst to preventing fusion of the phagosome-endosome (Alexander et al. 1999). The CDC life cycle below depicts the anthroponotic cycle of leishmaniasis. During zoonotic disease, there are additional animals involved from which the sand fly can take blood and acquire infection prior to infecting a

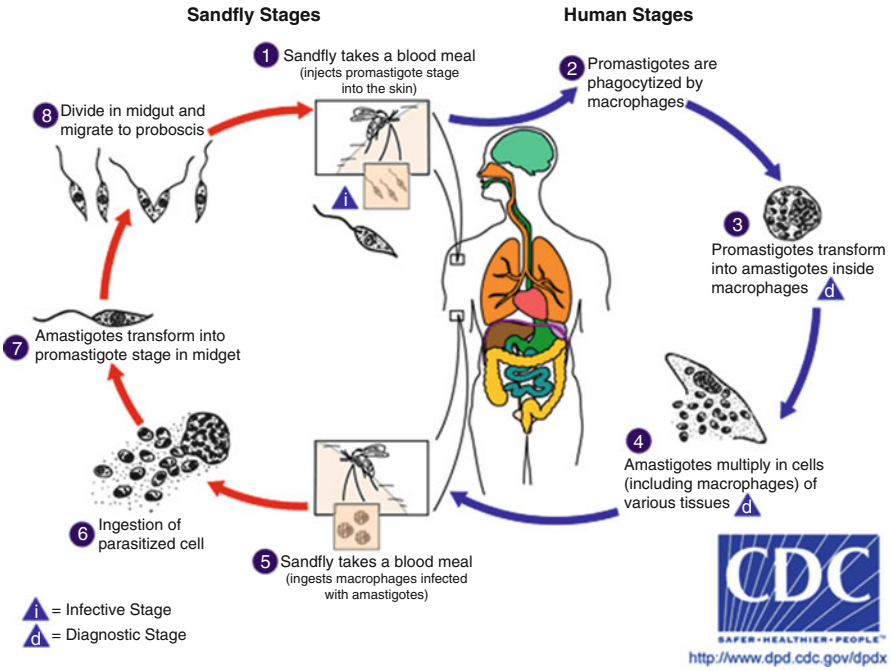

Fig. 4.5 Transmission cycle of *Leishmania* parasites. "Leishmaniasis is transmitted by the bite of infected female phlebotomine sandflies. The sandflies inject the infective stage (i.e., promastigotes) from their proboscis during blood meals (*1*). Promastigotes that reach the puncture wound are phagocytized by macrophages (*2*) and other types of mononuclear phagocytic cells. Progmastigotes transform in these cells into the tissue stage of the parasite (i.e., amastigotes) (*3*), which multiply by simple division and proceed to infect other mononuclear phagocytic cells (*4*). Parasite, host, and other factors affect whether the infection becomes symptomatic and whether cutaneous or visceral leishmaniasis results. Sandflies become infected by ingesting infected cells during blood meals (*5*, *6*). In sandflies, amastigotes transform into promastigotes, develop in the gut (*7*) (in the hindgut for leishmanial organisms in the *Viannia* subgenus; in the midgut for organisms in the *Leishmania* subgenus), and migrate to the proboscis (*8*)." Republished with permission from the CDC http://www.cdc.gov/parasites/leishmaniasis/biology.html

person. Other modes of transmission that have been documented include mother to fetus, organ transplantation, sharing needles, blood transfusion, sexual transmission, and acquisition during laboratory work by needle stick injury (Singh 2006) (Fig. 4.5).

5 Vector

The sand fly is the vector responsible for transmission of *Leishmania*. Of the nearly 800 recognized species of sand flies, 70–80 species are known to transmit disease (Cook and Zumla 2009). Sand flies belong to the subfamily *Phlebotominae*. There are two

genera of phlebotomine sand flies responsible for transmitting disease to humans, *Phlebotomus* in the Old World and *Lutzomyia* in the New World. There are wide variations in preferred habitats and breeding sites among species, with transmission occurring in regions as varied as arid climates, tropical rain forests, and urban dwellings (Goddard 2000). In India, studies have indicated that sand flies are present in highest number when the temperatures are highest and rainfall is lowest. They are almost nonexistent during the colder winter months in Northern India and populations tend to decrease during monsoon season. *Lutzomyia* tend to live in forest habitats in the New World. Recognition of breeding sites and seasonal patterns becomes extremely important in implementing effective vector control strategies (Picado et al. 2010a,b,c).

In some instances, there seems to be an extremely close specificity between vector and parasite. In such a case, the sand fly is considered restrictive, likely resulting from a long co-evolutionary process. Flagellates were found in sand flies dating back to the early cretaceous period (Ramalho-Ortigao et al. 2010). For example, *Phlebotomus papatasi* will only carry *Leishmania major*, despite ecologic overlap into areas harboring other species of *Leishmania*. Other sand flies are considered permissive and can experimentally become infected with several species of *Leishmania*, such as *Lutzomyia longipalis* which can transmit *L. infantum* or *L. mexicana* (Ramalho-Ortigao et al. 2010).

The sand fly life span is decreased by *Leishmania* infection and there are studies documenting affect on fecundity and others showing no effect. In addition, there is documentation that the sand fly attempts to mount an immune response to *Leishmania*. Certain sand flies make defensins and serpins as an immune response, digestive enzymes may be up-regulated, and the sand fly may induce apoptosis of midgut cells to make adhesion more difficult for the parasite (Ramalho-Ortigao et al. 2010; el Sawaf et al. 1994).

The sand fly life cycle begins with the female laying eggs (usually 30–200) after engorgement from a blood meal. The female sand fly lays her eggs in breeding sites which are usually specific to the type of sand fly and the ecological niche in which they live. There is a wide range of breeding site preferences and for many sand flies the breeding sites have not been elucidated. Usually breeding sites occur in areas where there is a presence of moist soil rich in organic matter, often in close proximity to blood meal hosts for newly emergent females. Recognized breeding sites in the New World have been associated with rain forest floors, contaminated soil of animal shelters, rodent burrows, and tree root buttresses. In the Old World, breeding sites tend to occur in cow sheds, earthen floors of human habitations, rodent burrows, caves and rocks, and termite burrows (Feliciangeli 2004). Eggs hatch as the 1st larval instars complete four larval instar stages in 30–60 days before pupation. Pupation lasts 7–8 days and ends with a newly emergent sand fly. Males usually emerge prior to females and seek a sugar meal. Females emerge next and take a sugar meal prior to searching for a blood meal (Eldridge and Edman 2000; Cook and Zumla 2009).

Male sand flies eat sugar meals provided by plants. Most females take blood meals for egg maturation but are not only hematophagous. They additionally eat sugar meals between blood meals. Upon biting the host, the sand fly's saw-like mouth causes local tissue destruction and blood pooling. Because of the nature of the bite, it is often

described as painful (Eldridge and Edman 2000; WHO 2010). Sand fly saliva causes vasodilation and prevention of clot formation at the site of the bite. The saliva has been shown to have immunomodulatory effects, differing depending on the sand fly species involved, including decreasing macrophage nitric oxide (NO)-dependent killing, inhibition of T-cell activation, inhibition of delayed-type hypersensitivity response, decrease in TNF-alpha and induction of IL-6 and IL-10. In experimental models of cutaneous leishmaniasis, saliva led to a higher parasite burden and greater lesion size (Hall and Titus 1995; Farrell 2002). Sand fly saliva induces potent vasodilation which recruits macrophages and other cells to the area, giving *Leishmania* an advantage in having cells to quickly infect. The saliva also modulates the cytokine profile and macrophage functions, making it more likely that infection will ensue (Hall and Titus 1995). Interestingly, upon reexposure to the same sand fly salivary proteins, there is experimentally the production of a delayed-type hypersensitivity reaction which leads to inherent immunity to reinfection (Reed 2001; Kamhawi et al. 2000).

Sand flies do not fly well. Their movement is described as "hopping," during which time they take small flights with periods of rest in between. They are usually found within 200 m of their breeding sites but have been found as far as 2 km away (WHO 2010). The sand fly life span is related in large part to climate and availability of sugar meals (Schlein and Jacobson 1999). Longevity has been found experimentally to be affected by *Leishmania* infection as well (el Sawaf et al. 1994).

6 Immunology and Pathogenesis

There is a considerable amount still to understand regarding *Leishmania* pathogenesis. The immune system and host genetic factors are thought to play a significant role (Blackwell et al. 2009). There has been considerable research in mice, but there are limited studies evaluating pathogenesis in people. There is additionally interspecies variation in pathogenic mechanisms. For example, mice known to be resistant to *L. major* are susceptible to *L. amazonensis* (Soong et al. 1997). Once *Leishmania* parasites are transmitted to the host by a sand fly, skin macrophages and dendritic cells phagocytose the parasites. The parasites are capable of using elaborate mimicry to hide themselves from immune destruction (Roberts et al. 2000). In cutaneous and mucocutaneous forms, the infection and resultant pathology will stay localized to the skin and mucosal regions. Interestingly, in *Leishmania braziliensis* infection, fever and systemic symptoms may be present prior to localization to skin or mucosal tissue with dissemination described through blood and lymphatics to the mucosa (David and Craft 2009; Ahluwalia et al. 2004). In visceral disease, the pathology is not localized to skin and mucosa but disseminates to the mononuclear phagocytes and reticuloendothelial system throughout the body. Areas most affected include the spleen, bone marrow, and lymph nodes (Mandell et al. 2010). In the liver, infected macrophages produce cytokines which cause destruction to liver tissue (Dias Costa et al. 2007). Aside from the parasite's affinity for causing cutaneous, mucocutaneous, or visceral disease, the human immune system plays a role in influencing the manifestations and severity of disease. There may additionally be a genetic role

assisting in this determination (Hsieh et al. 1995; Jeronimo et al. 2007). Expression of a Th1 pathway has been shown to be protective in infection with *L. major* whereas Th2 expression increases susceptibility. Other research has indicated that tumor necrosis factor-alpha and beta (TNF-alpha and beta) (Roberts et al. 2000) production increase the risk of development of mucosal disease. Investigation of skin biopsy samples for the presence of cytokines in localized cutaneous versus mucosal disease found increased levels of interleukin-4 (IL-4), IL-5 and IL-10 in mucosal disease without significant difference exhibited in other cytokine profiles, indicating perhaps a mixed Th1 and Th2 response (Pirmez et al. 1993). Another study of mucosal disease showed a mixed Th1 and Th2 profile, favoring higher Th1 cytokine levels. The patients who did not relapse had higher IL-10 levels pre-treatment (Tuon et al. 2008a,b; Soong et al. 1997). Studies challenging mucosal leishmaniasis patients' periperhal blood mononuclear cells (PBMCs) with *Leishmania* antigen showed increased production of interferon-gamma (IFN-gamma) and tumor necrosis factor-alpha (TNF-alpha) with a down-regulation in IL-10, proposing that the increased inflammatory response is likely worsening disease manifestations (Bacellar et al. 2002). In a study stimulating PBMCs to evaluate cytokine differences in patients either with active *L. infantum* (synonymous with *L. chagasi*) visceral leishmaniasis, asymptomatic disease and cured disease, it was seen that the predominance of Th2 response led to active disease, a balance of Th1 and Th2 was necessary for asymptomatic infection and Th1 predominance became important in patients with established cure (Peruhype-Magalhães et al. 2005). Prior studies showed that neutrophils were important in limiting splenic burden and depletion of neutrophils in mice contributed to increased parasitic growth (Peruhype-Magalhães et al. 2005).

7 Clinical Disease Manifestations

7.1 Cutaneous Leishmaniasis

7.1.1 Old World

Cutaneous leishmanaisis occurs in both the Old and New World and cumulatively accounts for the greatest number of cases of leishmaniasis worldwide. Old World and New World leishmaniasis tend to present differently and often have a different natural disease courses. In Old World cutaneous leishmaniasis, lesions usually start as nodules or papules at the site of the sand fly bite. The lesions continue to grow over the period of a week (WHO 2010) and will often develop an encrustation. Parasitic species differ between regions, as do different zymodemes within a species. This, in addition to host nutritional status, genetics, and immune status, and size of inoculation are often responsible for different presentations of disease (WHO 2010; u Bari et al. 2010; Calvopina et al. 2006). Although disease most commonly presents in a certain manner, clinical evaluation cannot be used solely to determine species, necessary treatment, and predicted clinical outcome.

7.1.2 New World

New World cutaneous leishmaniasis is caused by a number of species within both subgenera, *Leishmania* and *Viannia*. There is often formation of a macule or papule progressing to ulceration or nodularity (WHO 2010). After infection, the time to manifestations is variable, ranging from weeks to years (WHO 2010). In the subgenera *Viannia*, there may be associated lymphadenopathy (WHO 2010).

There are a number of other clinical manifestations of the disease other than localized cutaneous leishmaniasis.

Diffuse cutaneous leishmaniasis is most often seen with *L. amazonensis*, *L. mexicana*, and *L. aethiopica* (now known to be *L. donovani*). In HIV populations, it has additionally been seen with *L. braziliensis*, *L. infantum*, and *L. major*. Diffuse cutaneous disease is characterized by macules, papules, nodules, plaques, and skin infiltration with widespread manifestation. Ulcerations are atypical. The disease is similar in the Old and New World and frequent relapses are seen after completion of therapy. Once the disease is firmly established, achieving clinical cure is unlikely (Morrison et al. 2010). Occasionally Old World cases may have mucosal involvement located in close proximity to the mouth and nostrils. There is a lack of antigen-specific cell-mediated immunity, creating an anergic state in which the parasite can proliferate and cause diffuse disease (Morrison et al. 2010; Barral et al. 1994). Often the Montenegro skin test, a test looking for delayed-type hypersensitivity to *Leishmania* is negative because of the anergic response to the parasite despite presence of anti-*Leishmania* IgG antibodies (Barral et al. 1994; Silveira et al. 2004).

Disseminated cutaneous leishmaniasis presents with multiple scattered papular or ulcerated lesions. Nodular lesions are uncommon, a difference from diffuse disease (Carvalho et al. 1994). The most common causative species is *L. braziliensis*. Other species which have occasionally been noted to cause disseminated disease include *L. panamensis*, *L. guyanensis*, and *L. amazonensis*. The disease is difficult to treat and full cure is rarely achieved (WHO 2010). Montenegro skin tests remain positive with this form of disease but there is thought to be a cell-mediated immune defect that normalizes after treatment (Costa et al. 1986). Up to 38% of cases of disseminated disease have associated mucosal involvement (Ogawa et al. 2006; Carvalho et al. 1994). One study indicated lower levels of INF-gamma and TNF-alpha in patients presenting with disseminated cutaneous leishmaniasis as opposed to those with localized disease (Leopoldo et al. 2006).

Leishmaniasis recidivans is another described presentation of cutaneous leishmaniasis. The disease is most often associated with *L. tropica* and is less often seen with *L. braziliensis* (Dedet and Pratlong 2009). The disease is characterized by a paucity of amastigotes present in tissue, making accurate diagnosis challenging. The parasites are usually cleared in the center of the lesion with outward expansion of newly infected tissue. The lesion is slow growing and over years can cause disfigurement, with lesions occurring most commonly on the face. The condition is poorly responsive to treatment (Sharifi et al. 2010).

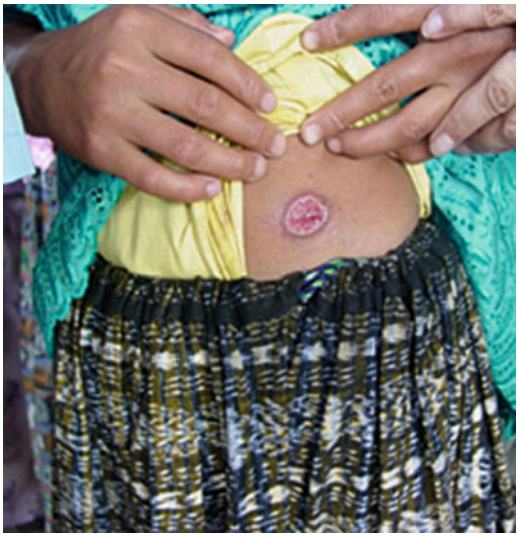

Fig. 4.6 Typical New World cutaneous leismaniasis ulcer. Photo courtesy of B. Arana, MERTU, Guatelmala

7.2 Mucocutaneous Leishmaniasis

Mucocutaneous leishmaniasis is a devastating illness found more commonly in the New World, though Old World species have been clearly implicated as well. The most common species causing true mucocutaneous disease is *L. braziliensis*. Other less common causative species include *L. panamensis*, *L. guyanensis*, *L. major*, *L. tropica* and *L. infantum* (Reithinger et al. 2007; Osoria et al. 1998). In Brazil, the disease is called espundia. The disease is caused by hematogenous or lymphatic spread to mucosa and can result in facial deformation, laryngeal obstruction, and pharyngeal destruction. The disease most often begins with the appearance of a typical cutaneous lesion. The mucosal involvement usually commences months to years after the primary lesion has healed (WHO 2010). There are exceptions, however, including mucosal involvement occurring concomitantly with the primary lesions as well as disease occurring as late as 30 years after healing of the primary lesions (Roberts and Janovy 2009; Reithinger et al. 2007; Magill 2010; Osoria et al. 1998). Interestingly, in certain patients, *L. braziliensis* is associated with constitutional symptoms such as fever, malaise, anorexia as well as hepatosplenomegaly and lymphadenopathy at the time of the initial skin lesion (Sousa Anastacio de et al. 1995; Barral et al. 1995a,b). Five to twenty-five percent of patients with resolved localized cutaneous lesions in endemic areas develop mucocutaneous leishmaniasis (Reithinger et al. 2007; Ahluwalia et al. 2004). Patients with mucocutaneous disease manifestations tend to have higher circulating levels of TNF-alpha, thought to be due at least in part to allelic differences predisposing to higher levels of TNF-alpha (Blackwell 1999). In addition, new research suggests that presence of *Leishmania* RNA virus infecting the *Leishmania* parasite may worsen the virulence of *Leishmania* (Ives et al. 2011).

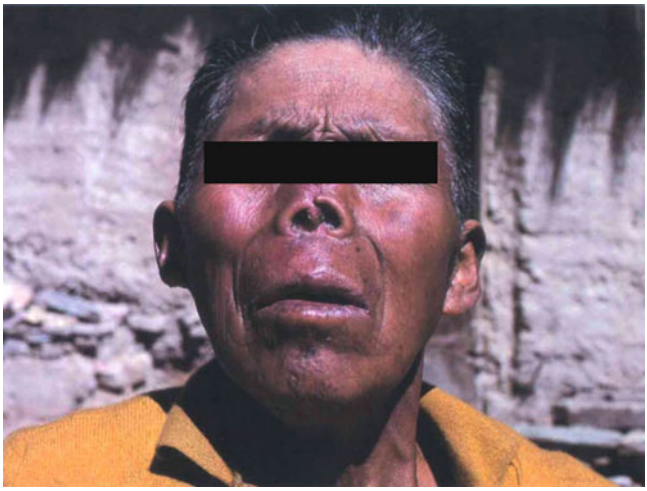

Fig. 4.7 Man with mucocutaneous leishmaniasis. *Source*: http://www.paho.org/English/AD/DPC/CD/leish-fotos2.htm

The most common presenting symptom of mucocutaneous leishmaniasis is nasal stuffiness early in the course of disease. As the disease progresses, lesions can cause airway obstruction, tracheal involvement, fibrosis causing inability to eat and secondary bacterial infections are commonly encountered, including aspiration pneumonia (Reithinger et al. 2007; Ahluwalia et al. 2004; Lawn et al. 2004). In many parts of the world, the significant facial deformations can be socially devastating. The disease is difficult to treat and continues to progress without treatment. Even with treatment, relapse can occur (Amato et al. 2009).

7.3 Visceral Leishmaniasis

Visceral leishmaniasis develops when hosts are infected with viscerotropic species of *Leishmania*, including *L. donovani* and *L. infantum* (syn with *L. chagasi* in the New World). There are rare cases caused by *L. tropica* (WHO 2010) and *L. amazonensis* (Magill 2010). There is a subset of patients who have subclinical disease and infection is not recognized in these patients (Badaro et al. 1986). The symptoms of visceral leishmaniasis usually begin with fever and lassitude with an atypical fever pattern, the most common variation occurring twice per day (Cook and Zumla 2009, p. 101; Most and Lavietes 1947). The acute presentation seen in malaria is notably absent with rare exceptions in nonimmune populations (Magill 2010; Most and Lavietes 1947). In areas where disease in endemic, children are most commonly affected as they have not yet acquired immunity. Adults are most commonly affected in epidemic settings, when malnutrition is frequent (affecting immunity), in areas of high HIV prevalence and when leishmaniasis is new to a particular population.

Because the disease occurs in areas where malnutrition and HIV are common problems, the disease is becoming an even greater concern.

The disease incubation period is usually 2–6 months (Chappuis et al. 2007) though disease has been known to manifest more than 20 years after initial infection (Uzair et al. 2004). The disease targets mononuclear phagocytes and the reticuloendothelial system resulting in marked splenomegaly followed by hepatomegaly. Transaminitis and portal hypertension progressing to development of varices can occur (Prasad et al. 2010). Bone marrow involvement occurs and can result in further leukopenia, anemia, and thrombocytopenia. The immune response leads to hypergammaglobulinemia. Proteinuria is a common finding and a spectrum of glomerular and tubular manifestations can occur (Elnojomi et al. 2010; Most and Lavietes 1947). Common signs and symptoms include fever, fatigue, and wasting. Once clinical infection ensues, there is continued progression without treatment until death occurs, usually as a result of secondary infection or bleeding, with the exception of rare instances in which spontaneous resolution occurs. In India, darkening of the skin occurs and is named kala-azar as a result. Secondary infection and bleeding are the most common causes of death.

7.4 Post-Kala-Azar Dermal Leishmaniasis

Another dermal manifestation of leishmaniasis is post-kala-azar dermal leishmaniasis (PKDL). This disease follows clinical resolution of visceral leishmaniasis and is characterized by the development of multiple macular, papular, and/or nodular lesions of the skin without ulceration. In Sudan, the disease can occur concurrently with visceral leishmaniasis up to one year post cured infection with 50–55% of cases presenting within six months of initial disease (Thakur et al. 2008; Zijlstra and el-Hassan 2001). In India, it is more common that disease is not seen until one year post-treatment and may not present until 20–30 years after clinical cure has been apparently achieved. There are several proposed mechanisms for development of PKDL. It is thought that parasites evading treatment may preferentially go to the skin to further establish infection. In Sudan, however, the disease can occur concurrently and it is hypothesized that perhaps genetic parasitic differences may enhance skin tropism. Additionally, different immune responses are seen in different severities of PKDL with increased IL-10 and TGF-β correlating with increased severity of disease. Increased levels of these also persisted in patients treated with pentavalent antimony that went on to develop PKDL (Saha et al. 2007). There seems to be a lack of specific cell-mediated immunity that returns after treatment. Another observation is that the incidence seems to be decreasing in areas where amphotericin was used as first-line treatment for visceral leishmanaisis rather than antimonials. Interestingly, the two drugs seem to induce differences in cytokine profiles, so despite an increased incidence of kala-azar, PKDL seems to be decreasing in India where antimonials are being used less often. Because miltefosine has excellent skin penetration, it was unknown whether PKDL would develop after treating visceral leishmaniasis (VL)

with miltefosine. Recent case reports, however, document the occurrence of PKDL after VL treatment with miltefosine (Das et al. 2009). Approximately 5–10% of patients in India and 50–60% of patients in Sudan and East Africa surviving visceral leishmaniasis will develop post-kala-azar dermal leishmaniasis (Ganguly et al. 2010). Macrophages harboring amastigotes are found within the skin with the highest density seen in nodular lesions (Ganguly et al. 2010). In Sudan, the disease usually self-heals and treatment is commonly administered after one year of persistence. In India, the disease does not self-heal. Because the skin lesions lead to a continued reservoir of infection, there has been increased advocacy to treat the disease early to prevent further case transmission (Ganguly et al. 2010).

8 Diagnosis of Leishmaniasis

Diagnosis of cutaneous leishmaniasis can be accomplished by a number of methods. Preferred methods are often dictated by available resources, despite suboptimal sensitivity or specificity. In most areas where leishmaniasis is endemic, resources are not available for complex molecular laboratories where PCR can be adequately performed. The most commonly employed means of diagnosis is direct tissue examination using light microscopy of Giemsa-stained tissues. Using this method, sensitivity is dependent upon the parasite load and the tissue sampled. Specificity for direct microscopy is 100%. Sensitivity ranges from 15–30% in the New World to 50–70% in the Old World (Goto and Lindoso 2010). Tissue can be obtained by a number of methods including swabbing the lesions, fine needle aspiration, or punch biopsy. Punch biopsy is the preferred method of tissue collection when parasite density is sparse.

Histopathology of prepared tissue sections can additionally be utilized as a method for diagnosis, limited again by similar factors such as tissue sampled and parasite density. In areas where it is possible to be infected with more than one species of parasite, these methods are disadvantageous as they cannot ascertain a particular species. This is important given the different response to treatments and the different clinical behavior of certain parasitic species. Culture using media can allow for speciation but this requires more advanced laboratory resources and sensitivity is lacking. PCR is presently considered the best diagnostic method when resources permit given its ability to correctly identify the parasite down to the species level with the best sensitivity and specificity.

Serologic tests in the form of ELISA, direct and rapid agglutination tests, nanoparticle biosensors and others (Perinoto et al. 2010) are employed not infrequently in the field but have the distinct disadvantage of being unable to differentiate between acute and prior infection. Additionally, titers may be low and difficult to detect in cutaneous disease. When titers are present, they may be present for years. There can be cross-reactivity with other organisms (Romero et al. 2009; Singh and Sivakumar 2003). However, in instances when the clinical presentation is consistent, this method is sometimes used because of its ease of use, simple technique, and affordable cost. Diagnostic information is sometimes obtained from the Montenegro skin test,

also called the leishmanin skin test, a test that evaluates for delayed-type hypersensitivity response. It is most often used in epidemiologic studies and cannot differentiate between active and past disease and is often negative in diffuse cutaneous disease. It may be helpful in certain forms of cutaneous leishmaniasis.

Newer sampling modalities involve using filter paper to wick the lesion to obtain PCR substrate, decreasing the need for invasive sampling (Boggild et al. 2010). This method has a sensitivity of 92% and specificity of 100%. Even though the technique may be limited by need for PCR to be performed in a reference laboratory, the filter paper lends itself to field collection of samples which can then be sent to a reference laboratory.

The gold standard for diagnosis of visceral leishmaniasis is microscopic visualization of Gimesa-stained amastigotes from splenic aspirates or bone marrow (Sundar and Rai 2002). Splenic aspirates not only yield the greatest results but also pose a greater risk of complication. Splenic biopsy can have grave consequences including death. In experienced centers, however, the rate of complications is extremely low (Sundar and Rai 2002). Amastigotes have been visualized in other tissues including lymph nodes and liver aspirates (Sundar and Rai 2002). PCR can be helpful in diagnosis but adequate laboratory facilities are necessary. PCR allows for speciation and can be done on filter paper blood spots as well as peripheral blood and bone marrow aspirates (Meredith et al. 1993; Sundar and Rai 2002; Srivastava et al. 2011a,b). Whole peripheral blood PCR, developed for clinical use, has a sensitivity and specificity of 88% and 84%, respectively, and other reports indicate sensitivity of PCR in blood of 98.5% and 95.6% in bone marrow aspirates (Srivastava et al. 2011a,b). Serology can be used to assist in diagnosing visceral leishmaniasis when other diagnostic modalities are not available. Depending on the tests used, antigens or antibodies can be detected. Antibody tests cannot determine acute versus prior disease given the longevity of circulating antibodies after cure of the disease (Sundar and Rai 2002). The rK39 immunochromatographic strip test is one of the more commonly used field tests to aid with serologic diagnosis of visceral leishmaniasis caused by *L. donovani* and is helpful when paired with the correct constellation of signs and symptoms suggestive of visceral leishmaniasis. Direct agglutination test (DAT) and the rK39 test were compared and had similar sensitivity and specificity (Sundar et al. 2006a,b,c). Skin testing is not useful in acute diagnosis as the patients are negative until cured, at which point they develop a positive skin test (Sundar and Rai 2002).

8.1 Treatment

8.1.1 Visceral Leishmaniasis

Visceral leishmaniasis (VL) is a devastating disease causing death in most untreated cases. There are a number of effective drugs for treating VL, usually limited by resources in affected areas. Pentavalent antimonials have been the mainstay of treatment for visceral leishmaniasis for several decades. There has been a push to

evaluate other drug treatments because of the increasing resistance and growing inefficacy of these drugs, especially in India, the most endemic location for visceral leishmaniasis (Bryceson 2001; Sundar 2001). More recently, paromomycin, multiple formulations of amphotericin B and miltefosine have been used for treatment, with a growing trend supporting multidrug treatment in an effort to curb development of drug resistance and decrease overall side effects. There are a number of other treatments found to have some efficacy as well but are either less efficacious or have a poor side effect profile, which has mostly eliminated their use.

Amphotericin B has been studied in traditional and in lipid formulation in many different dosage regimens. The lipid formulations have a lower side effect profile but at a higher cost with similar efficacy to that seen with amphotericin B deoxycholate (Sundar et al. 2004). Short courses of treatment with liposomal amphotericin B have been successful for both *L. infantum* and *L. donovani* (Davidson et al. 1996). One appealing study looked at single dose liposomal amphotericin with 95% efficacy seen in the study. There is concern that parasite resistance could develop with this short regimen if parasite clearance is not fully achieved (Sundar et al. 2010). Subsequent studies again showed excellent efficacy following single dose amphotericin with a short course of oral miltefosine (Sundar et al. 2008). Because of the proven efficacy with various formulations of amphotericin, it has become the treatment of choice in India where antimonial drug inefficacy is problematic. While the majority of studies exclude patients with severe illness, Ambisome was additionally effective in field conditions in difficult to treat populations (Seaman et al. 1995) with cure rates of 88% using a 3–5 milogram per kilogram (mg per kg) regimen consisting of six doses spread over a 2-week period.

Miltefosine has become a promising drug because it can be administered orally, negating the need for costly hospitalization. A published study in 2006 showed efficacy similar to amphotericin B (Sundar et al. 2006a,b). Miltefosine achieved 6-month cure rates of 100% with 28 days of treatment with efficacy decreasing to 89% with a 14-day course (Sundar et al. 2000a). Because of the lack of need for hospitalization and decreased cost of the drug, the drug is presently being used as a treatment modality (Sundar et al. 2006c). Paromomycin has shown excellent efficacy in India as well, where 95% of patients were successfully treated with paromomycin (Sundar et al. 2009a,b). The cure rates were significantly different in East Africa. A regimen of 15 mg per kg for 21 days, the same regimen found successful in India only lead to a 50% cure rate of Sudanese patients after 6 months (Hailu et al. 2010). The study was repeated, extending the treatment duration to 28 days, which improved efficacy to approximately 80% (Musa et al. 2010). Because of the marked difference in efficacy by region, it has become clear that study results cannot be generalized to encompass treatment recommendations for all patients with visceral leishmaniasis. Each drug needs to be studied independently in each region to gain adequate treatment data.

Pentavalent antimonials are still considered first-line therapy in many areas of the world, especially in Sudan where clinical trials have not clearly shown improved efficacy over other modalities (Hailu et al. 2010). In these regions, the decrease in efficacy has not been demonstrated as it has in India and Nepal (Rijal et al. 2010). In areas where pentavalent antimonials were used inappropriately, there was a

significant rise in unresponsiveness to the drugs. Drugs were often started at low doses and increased over a week's time and treatment interruptions were frequently observed, both of which have contributed to drug resistance. Often, drugs were prescribed by providers with limited knowledge regarding the specific medications and correct dosages necessary to treat kala-azar. In one survey, only 26% of patients were treated according to recommended drug standards (Croft et al. 2006). Decreased sensitivity was seen by amastigotes in nonresponders. It is thought that increasing the drug dosage may overcome the decreased sensitivity. However, resultant increased drug side effects preclude use of an increased dosage. It has additionally been observed that unresponsiveness is often geographically specific. For instance, in regions in Bihar, India, unresponsiveness over the years has steadily worsened, with efficacy dropping to 40% in some areas (Sundar 2001) while 200 miles away, drug responsiveness is preserved in 97% of cases. In Nepal, risk factors for treatment failure include fever or greater than12 weeks duration, prolonged fever, treatment interruption, ambulatory treatment and living close to Bihar's high resistance regions (Rijal et al. 2010).

Immunomodulatory Treatment

Because of the recognition that there is a need for Th1 response to assist in parasitic clearance, studies have been done in mice using antiparasitics in combination with immunomodulators to improve parasite clearance, with promising effects (Banerjee et al. 2011).

Multidrug Treatment

Because of the high levels of drug nonresponse which has continued to worsen in India, at least in part due to drug resistance, recent trials have looked at the efficacy and safety of shorter courses of multiple drugs to treat the disease. The rationale is several-fold. Firstly, the hope is that drug resistance will be less likely to develop when multiple drugs are used concurrently. Secondly, the duration of drug exposure is usually less in combination therapy, which tends to decrease the prevalence of side effects. Thirdly, the cost should be decreased as each drug is needed for a shorter period of time, costly hospitalization can be avoided, and tests monitoring for side effects may no longer need to be performed. One recent trial evaluated the current standard of care in India, amphotericin B 1 mg per kg every other day, to combinations of (1) single dose amphotericin at 5 milligrams (mg) per kilogram (kg) for 7 days in addition to miltefosine 50 mg orally for 7 days, (2) single dose liposomal amphotericin B at 5 mg per kg in addition to 10 days of IM paromomycin at 11 mg per kg and (3) 10 days of combined miltefosine and paromomycin at the dosages noted above. In all arms, the response rate was excellent with 93% efficacy in the amphotericin group compared with 97% in the combination groups. There were fewer side effects in the combination arms (Sundar et al. 2011; Meheus 2010; van Griensven and Boelaert 2011).

9 Post-Kala-Azar Dermal Leishmaniasis

There are a number of treatments which have been used for PKDL. Pentamidine has been successfully used in treatment but is usually avoided due to significant development of side effects. Ketoconazole has also been used at a dose of 800 mg per day for 9 months. Allopurinol likely has some effect, but took 20–24 months for effect to be seen (Zijlstra et al. 2003). More commonly employed treatments are amphotericin B and pentavalent antimonials. In a trial comparing antimonials and amphotericin B in India, 11 of 11 patients were cured with three cycles of amphotericin while 7 of 10 were cured with sodium antimony gluconate (SAG) requiring 6–10 cycles to attain cure (Thakur et al. 1997). Six doses of Ambisome at 3 mg per kg successfully treated an HIV PKDL coinfected patient who did not respond to a long course of miltefosine, though the study interpretation is difficult as the patient was on and off highly active antiretroviral therapy (HAART) during the miltefosine portion of treatment (Guffanti et al. 2008). Another case report found that a patient who did not respond to sodium stibogluconate (SSG) responded instead to 100 mg per day of miltefosine in divided doses for 12 weeks (Sundar et al. 2006a,b). A study in Sudan evaluated patients with persistent PKDL and found an 83% cure rate in patients given 3.5 mg per kg per day of Ambisome for 20 days (Musa et al. 2005). In India, it was reported that three patients were successfully treated with 8 weeks of 50 mg per kg of miltefosine (Khandpur et al. 2010). HIV patients with PKDL have also responded to miltefosine (Belay et al. 2006). Patients treated for PKDL with miltefosine had higher levels of interferon-gamma and CD40 transcripts after treatment than those treated with pentavalent antimonials (Ansari et al. 2008). Additionally, an immunosuppressed patient, status-post transplantation responded to Ambisome 100 mg per day for a total dose of 3 grams (g) (Roustan et al. 1998). Two patients previously treated for visceral leishmaniasis with miltefosine 50 mg twice daily for 28 days presented 4 years later with PKDL which responded to amphotericin B deoxycholate, requiring two or three 20-day courses at 1 mg per kg every other to achieve successful treatment. While there are multiple treatment modalities available, most of the literature is based on case reports and a clear treatment regimen has not been established.

9.1 Treatment of Cutaneous Leishmaniasis

9.1.1 Old World

In the Old World, because disease often self-resolves and there is not the same risk of progression to mucocutaneous disease as is seen with *L. braziliensis* in the New World, the mainstay of therapy has usually been topical treatment and sometimes, no treatment is initiated. In some cases, systemic treatment is indicated, depending to some degree on the species involved, the size of the lesion, number of lesions, and

the location of the lesions. When lesions are not too large or numerous, topical paromomycin or intralesional injections of antimony have been used (Babak et al. 2005). One study did not find improvement when pre-treating with cryotherapy (van Thiel et al. 2010). One study looked at intralesional stibogluconate with injections given on day 1, 3, and 5 of each monthly cycle. After three cycles, only 58.3% were cured. This was compared with the addition of ketoconazole for 4 weeks with a 93.3% cure rate at 12 weeks and also compared with intralesional injections in addition to one intramuscular dose of stibogluconate with resultant 93.3% cure rate at 12 weeks (El-Sayed and Anwar 2010; Sharma et al. 2005). When twice weekly sodium stibogluconate injections were given for 10 weeks for *L. tropica*, there was a 73% cure rate (Bumb et al. 2010). Combination with short course systemic medications seems to decrease systemic side effects but improve efficacy (Tallab et al. 1996). An Iranian study did not type species but looked at standard injections versus delivery via a mesotherapy gun for intralesional glucantime and found that the mesotherapy gun allowed for more precise delivery of medication. The cure rates were similar but there was less pain and fewer treatment sessions were needed for the mesotherapy gun delivery method (Kashani et al. 2010).

Systemic treatment is used more often when topical treatment has failed, large lesions are present, when multiple lesions are present, or when injections are not feasible based on location. Systemic therapies include amphotericin B, pentavalent antimony, miltefosine, imidazoles, and nitazoxanide (Gurgen et al. 2011; Solomon et al. 2010). Amphotericin distributes to the spleen and liver rather than the skin, suggesting potential decreased efficacy for cutaneous disease. There are anecdotal reports of success, however, using this agent (del Rosal et al. 2010; Wortmann et al. 2010). Failures have been seen in New World disease using Abelcet (Wortmann et al. 1998). Given its excellent skin penetration, miltefosine has been used as well. There are case reports of Canadian soldiers with *L. tropica* who failed fluconazole treatment, one was given sodium stibogluconate, and the others were given miltefosine and all attained cure (Keynan et al. 2008). Miltefosine was used in case reports in Old World disease treating *L. tropica* in Afghanistan (Killingley et al. 2009) and New World disease with *L. braziliensis* from Central America at a dose of 2–2.5 mg per kg daily for 28 days with resolution and no relapse at 4 months. The patients were not followed up for a long enough time frame to adequately look for relapse (Tappe et al. 2010). Imidazoles have been used as well for Old World disease. A child infected in Africa was treated with fluconazole 150 mg daily for 12 weeks with resolution (Sklavos et al. 2010). In addition, there are case reports showing prior cures with ketoconazole in cases from Israel, Algeria, Saudi Arabia, and Ethiopia (Ramanathan et al. 2011). One patient in the literature with *L. infantum* cutaneous disease responded to 14 days of posaconazole (Paniz Mondolfi et al. 2011).

9.1.2 New World

New World disease is viewed differently in terms of treatment. Because of the propensity for development of mucocutaneous disease with *L. braziliensis* and because of decreased likelihood of resolution of lesions, treatment is often

undertaken and systemic therapy is used more commonly than in the Old World. The available treatment modalities are similar to those presented earlier. However, small studies have shown differing results of therapies in different locations, and there is general agreement that treatment should be determined by local experience in each area given the profound regional and species differences determining effective treatment. This section will discuss some of the case reports and studies done in the New World.

9.1.3 Antimonials

Some of the initial antimony studies recommended a dose of 850 mg, later decided to be too low of a dose. Once the treatment dosages were increased to 20 mg per kg without a dosage cap for 20 days (15 days of treatment was found to be suboptimal), there was improved success (Herwaldt and Berman 1992). Antimony was compared to miltefosine at 2.5 mg per kg daily for 28 days versus parenteral antimony for 20 days for *L. guyanensis*. The cure rates were 71.4% in the miltefosine group and 53.6% in the antimony group (Chrusciak-Talhari et al. 2011). A Peruvian study looked at the different response rates to antimonials by species. *L. braziliensis* had a 30.4% treatment failure, *L. peruviana* had a failure rate of 24.5%, and *L. guyanensis* responded well with a small 8.3% failure rate (Arevalo et al. 2007). *L. panamensis* in Panama had a cure rate of 68–76%, but the dosage in the study was capped at 850 mg daily (Herwaldt and Berman 1992). Studies evaluating duration of antimonials in soldiers returned from duty showed that 19 of 19 were cured in 10 days and 18 of 19 were cured in the 20-day group. The study looked at Old and New World cutaneous disease and did not specify exact location where infection was acquired. The species showing cure in the 10-day group were *L. panamensis*, *L. major*, *L. braziliensis*, *L. naiffi*, *L. guyanensis*, and *L. tropica* (Wortmann et al. 2002). In a significant number of studies, patients were only followed to evaluate for relapse for 6–9 months which may not be an adequate amount of time to look for relapse rates. In one meta-analysis, only 12 articles met criteria for analysis with an antimony cure rate of 76%. In this analysis, there was no differentiation between *L. braziliensis*, *L. amazonensis,* and *L. guyanensis*. In the same study, when antimonial treatment failed, pentamidine was superior, intravenous (IV) paromomycin was less effective, and in general there was not enough evidence to support allopurinol, topical paromomycin, IV paromomycin, and imidazole drugs (Tuon et al. 2008a,b). A study in Peru evaluated the risk factors for failure to pentavalent antimony and found an overall failure rate of 24.4% when using 20 mg per kg per day for 20 days. Risk factors for failure included age, stay of less than 72 months in area of acquisition, duration of disease under 5 weeks, additional lesions, and infection with either *L. peruviana* or *L. braziliensis*. (Llanos-Cuentas et al. 2008). In Brazil, studies evaluated the difference in antimony response between *L. braziliensis* and *L. guyanensis* using meglumine at 20 mg per kilogram per day for 20 days. The cure rates for *L. braziliensis* and *L. guyanensis* were 50.8% and 26.3% respectively (Romero et al. 2001). Another study looked at addition of a vaccine in combination with antimony

to improve treatment success rates. Treatment rates improved to 80% from 33% in the placebo group when using vaccine with pentavalent antimony (Llanos-Cuentas et al. 2010). Another study compared pentostam to ketoconazole in Guatemala (Navin et al. 1992). For *L. braziliensis*, there were cure rates by 52 weeks of 96% with pentostam, 43% with ketoconazole and 7% with placebo. For *L. Mexicana*, clinical cure was achieved for 70% of patients treated with pentostam, 81% of patients in the ketoconazole group, and 56% of placebo patients.

9.1.4 Miltefosine

A pilot study in Columbian soldiers indicated 94% efficacy in treatment groups receiving 133–150 mg per day for 3–4 weeks (Soto et al. 2001; Soto and Berman 2006). In another controlled Columbian trial, selected cases were typed and there was a 91% cure rate with 2.5 mg per kg per day for 28 days in *L. panamensis*. In Guatemala, however, overall cure was 53% with 33% cure for those typed as *L. braziliensis* and 67% for those typed as *L. Mexicana*, again highlighting the drastic differences between species and region in terms of treatment response (Soto et al. 2004; Soto and Berman 2006). A study was repeated in Columbian soldiers and species of *L. panamensis* and *L. braziliensis* had improved response to meglumine over miltefosine (Velez et al. 2010). In a Brazilian study (Machado et al. 2010) performed in Bahia, Brazil, there was a noted decrease in the efficacy of antimony ranging from 50% to 90%. They looked at oral miltefosine and pentavalent antimony in this patient population. Seventy-five percent of patients were cured with 2.5 mg per kg of oral miltefosine daily versus 53% in the pentavalent antimony group at 20 mg per kg intravenous for 20 days. Only 57.7% had a positive culture and only 41 of 90 were typed by PCR and found to be *L. braziliensis*. When looking only at culture positives, there was a 76% cure in the miltefosine group and a 47.8% cure in the other group. A case report of two patients showed success with miltefosine after amphotericin failure. One patient was from Spain (OW) and one was from El Salvador (Ramanathan et al. 2011).

In another randomized controlled trial in Brazil (Chrusciak-Talhari et al. 2011), patients were given either 2.5 mg per kg per day of miltefosine for 28 days or glucantime 20 mg per kg per day intravenously (IV) for 20 days. Cure rates at 6 months in the miltefosine arm were 71.4 and 53.6% in the antimony group. All parasites were speciated and most were *L. guyanensis*. Of the three patients with *L. braziliensis*, one failed treatment in each group. The one case of *L. lansoni* was treated successfully with miltefosine. The patients were only followed for 6 months, and there was evaluation for parasitic cure only if there were residual lesions present. In a Bolivian study, 94% of cases were noted to be *L. braziliensis*. Groups were given either 2.5 mg per kg miltefosine for 28 days or 20 mg per kg glucantime for 20 days. By 6 months, 88% were cured with miltefosine and 94% were cured with glucantime without a statistically significant difference (Soto et al. 2008).

9.1.5 Imidazoles

Imidazoles are not commonly used given other treatment options. There are case reports showing variable treatment responses. Two cases of *L. braziliensis* failed treatment with ketoconazole (Dan et al. 1986). There are additionally reported failures with *L. Mexicana* (Baum and Berens 1994) and *L. guyanensis* (Dedet et al. 1986). Three of ten patients with cutaneous leishmaniasis seen at the National Institutes of Health were diagnosed with *L. V. panamensis* and all were treated successfully with ketoconazole (Ramanathan et al. 2011). One study looked at efficacy of ketoconazole 600 mg per day for 28 days versus inadequate dosages of Pentostam (sodium stibogluconate) against *L. panamensis*. There was a 76% cure rate in the ketoconazole arm and 68% cure rate in the Pentostam arm (Saenz et al. 1990). In Guatemala, a controlled trial of Pentostam versus ketoconazole for cutaneous leishmaniasis (Navin et al. 1992) showed that patients with *L. braziliensis* responded best to Pentostam and those with *L. mexicana* responded more favorably to ketoconazole. A case report from a patient in Belize indicated successful treatment with 200 mg fluconazole daily for 6 weeks. The species was not typed (Antonovich and Callen 2005).

9.1.6 Amphotericin

Seven patients with *L. braziliensis* were given short course liposomal amphotericin B and compared retrospectively to cases of *L. braziliensis* treated with sodium stibogluconate. There were no failures or relapses in the liposomal amphotericin arm but 10 of 27 patients who received pentostam had relapse (Solomon et al. 2007). The study found that there were no failures when treated with amphotericin and fewer side effects were seen than in patients treated with sodium stibogluconate (SSG).

The USA has not approved the use of pentavalent antimony but has now approved use of amphotericin B. A study was undertaken to retrospectively review response to amphotericin for both Old World and New World cases. The 10 Old World cases were from Iraq and Afghanistan with infection from *L. major* and *L. tropica*. In ten New World cases, including cases from Peru, French Guiana, Honduras, and Columbia with strains of *L. braziliensis*, *L. guyanensis*, and *L. panamensis*, 84% responded to a 7 dose regimen of 3 mg per kg of amphotericin B deoxycholate given on days 1–5, 14, and 21. In the 16% that did not initially respond, they improved with 7 more doses for the case of *L. braziliensis* and 4 and 7 more doses for the two cases of *L. panamensis* (Wortmann et al. 2010). There are further case reports of liposomal amphotericin showing efficacy in adults and children with *L. braziliensis* and *L. infantum* (Brown et al. 2005; del Rosal et al. 2010).

9.1.7 Other Treatments

Pentamidine is not frequently used in the treatment of cutaneous leishmaniasis as it achieves second-line efficacy and has a poor side effect profile (Calza et al. 2001; Nacher et al. 2001; Goto and Lindoso 2010; Gonzalez et al. 2009). Allopurinol has been reported effective alone and as an adjunctive to other therapies but its current use is negated by a wide choice of better alternatives (D'Oliveira et al. 1997; Llanos-Cuentas et al. 1997; Martinez et al. 1997; Velez et al. 1997; Baum and Berens 1994). Topical paromomycin was found to be less efficacious than parenteral pentavalent antimonials and parenteral paromomycin was found to be equally effective to parenteral pentavalent antimonials in a meta-analysis for treatment of New World cutaneous disesase (Kim et al. 2009). Other methods employed for treatment when systemic therapy is not felt to be necessary include topical therapy, intralesional therapy, heat, cryotherapy, immunotherapy, and photodynamic therapy (Goto and Lindoso 2010; Jowkar et al. 2010; WHO 2010).

9.2 Diffuse Cutaneous CL

Diffuse cutaneous leishmaniasis is challenging to treat. There is a large parasite burden coupled with host anergy and frequently despite multiple treatment courses, the disease may continue to relapse. Pentavalent antimony has been the drug of choice with imidazoles, miltefosine, and amphotericin used in some cases as well. Case reports using amphotericin B have shown significant success and further investigation for this indication is certainly warranted (Morrison et al. 2010). One case report of a boy with diffuse disease with *L. Mexicana* starting at age 3 which continued to worsen over years despite treatment with meglumine and immunotherapy finally responded to treatment with amphotericin B at a dose of 1 mg per kg per day to a total dose of 1,750 mg with total resolution seen by day 50 (Morrison et al. 2010). Another patient with diffuse cutaneous leishmaniasis and AIDS was initially given amphotericin B then miltefosine then 52 glucantime injections over 2 months with final resolution and no relapse at 2 years, again illustrating the challenge of treatment (Perez et al. 2006).

9.3 Disseminated CL

Disseminated cutaneous leishmaniasis is additionally difficult to treat, especially in an immunosuppressed population. Two cases in an area endemic for *L. braziliensis* were treated with glucantime. One was lost to follow-up and the other was treated successfully after administration of a total of 7.5 g of glucantime (Ogawa et al. 2006). According to the World Health Organization (WHO), there should be at least partial response to antimonials and miltefosine (WHO 2010).

9.4 Mucosal Leishmaniasis

Recommendations have indicated antimonials as the drugs of choice for treatment of mucocutaneous disease. Studies show a wide range of efficacy beginning with a study showing only 10% response rate of *L. braziliensis* with a sodium stibogluconate dose of 20 mg per kg for 28 days in severe cases and 75% cure rate in mild cases. Pentostam cured 77% of patients with mucosal disease from *L. panamensis* (Herwaldt and Berman 1992; Saenz et al. 1991; Franke et al. 1990). In 59 Peruvian patients with mucosal disease, there was a 63% cure rate for antimony combined with allopurinol and a 75% response rate to pentavalent antimony alone (Llanos-Cuentas et al. 1997). Extending treatment with sodium stibogluconate out to 40 days did not improve outcome over 28 days of therapy, with response rates of 63% in both groups (Franke et al. 1994).

A study in Rio de Janerio indicated that 91% of cases with mild disease were cured with low dose antimony at 5 mg per kg per day for 30–45 days (Oliveira-Neto et al. 2000). A study in Bolivia showed 86% cure in patients with mild mucosal disease, 58% cure in those with severe disease with 2.5 mg per kg miltefosine, and 50% effectiveness in the patients who received amphotericin B every other day for 3 months (Soto et al. 2007). Pilot studies have shown success with amphotericin B colloidal dispersion and liposomal amphotericin B (Amato et al. 2007). The WHO presently recommends using one of a number of possible therapies including pentavalent antimony for 30 days, amphotericin B deoxycholate, liposomal amphotericin B, miltefosine, pentamidine as second-line treatment, or a combination of oral pentoxifylline plus pentavalent antimonials which has been shown to decrease the relapse rate (WHO 2010).

9.5 Leishmania Prevention and Control Methods

Past control efforts for malaria had a significant effect on leishmaniasis. Some continued residual spraying for a short time period for leishmaniasis based on this finding but in most cases, control efforts were halted once malaria efforts ceased, with a sharp increase in incidence of leishmaniasis. More recently, the governments of India, Bangladesh, and Nepal, under the direction of the WHO, signed a kala-azar elimination/reduction agreement with the intent of decreasing the incidence of kala-azar to less than 1 in 10,000 in these countries by 2015. An endemic area in Bihar, India, was studied to estimate prevalence. An average of 21.26 cases of visceral leishmaniasis per 10,000 population was found in this region (Das et al. 2010b).

9.5.1 Indoor Residual Spraying

There has been considerable recent work targeting prevention of leishmaniasis, especially in the areas of Nepal, Bangladesh, and India, where incidence was recently reported to be greater than 20 cases per 10,000 population (Mondal et al. 2009).

As a result, the World Health Organization (WHO) initiated government control programs for vector control in an attempt to decrease disease incidence in these high prevalence regions. Controlled trails have shown clear efficacy of indoor residual spraying (IRS) (Joshi et al. 2009) with 72.4% reduction in sand fly counts with IRS. A recent report following up on the progress of the governmental control program found several problems leading to lack of efficacy in the kala-azar elimination attempt (Chowdhury et al. 2011). The governments are spraying one of the two insecticides (DDT and deltamethrin) for indoor residual spraying twice per year in these regions. The study evaluated insecticide bioavailability, quality of the insecticide, insecticide concentrations with IRS, insect susceptibility, and vector densities before and after spraying. The results left much to be desired. In India, the mortality of *P. argentipes* exposed to sprayed walls for 1 h decreased with time so that there was <25% mortality at 5 months after spraying. The quality of DDT was suboptimal with only 75.3% of the necessary concentration being utilized. In Nepal, 100% of expected insecticide concentration was present prior to spraying. In one of the Nepalese regions, however, the wall concentration was only 6.92% of the target goal. In India, the village-mean wall concentration was 73% of goal. The insecticide susceptibility in India to DDT was only 54.2% and in Nepal was 97%. Vector densities were assessed at 4 weeks. In India there was some community wide reduction of *Phelbotomus argentipes* at 4 weeks after IRS. In the Nepalese region of Sunsari, where concentrations were found to be low, the vector impact was short-lived, with the effect lasting only 2 weeks. In Sharlahi, there was impact present at 4 weeks. Further follow-up could not be accurately performed given monsoon flooding in India decreasing the overall sand fly rates and lime plastering in Nepal which also decreased the overall vector density. Though indoor residual spraying has been found effective in controlled studies, because of the many variables working in concert to lead to a successful program, the desired effect is currently lacking.

In Bangladesh, one of the three countries entering an agreement in 2005 to decrease the kala-azar incidence by 2015, one study found that the funds allocated for vector control for kala-azar were not being used for this purpose. The prevalence of disease was estimated to be 13%. Ninety-percent of homes had bed nets as part of malaria control (Mondal et al. 2008). When compared with the use of bed nets and lime plastering for prevention of leishmaniasis, IRS has been shown overall to be the most effective and least costly prevention method, though the cost of the different methods ranges considerably by region, making bed nets most cost-effective in Bangladesh (Joshi et al. 2009; Das et al. 2008). A study comparing the most effective methods for vector control in Nepal found that IRS was the most effective (Das et al. 2010a,b).

9.5.2 Active Case Finding

Active case finding may be an effective tool in identifying those in need of treatment. By treating patients earlier, outcomes are improved and there are decreased numbers of reservoirs to propagate further disease. In many areas, significant parts of the

population are still unaware of signs and symptoms of kala-azar and may not attribute symptoms to kala-azar. Many, when symptoms are present, seek care from providers untrained in providing adequate kala-azar treatment. By active case finding, on average 22.7 cases per 10,000 people were identified. In Muzaffarpur, India, the lowest newly diagnosed cases were found because of the prominent and trusted leishmaniasis treatment center which has led to improved diagnosis, treatment, and education about the disease. In Bangladesh, the population was least educated about the disease. On average, 267 houses needed to be evaluated for each case of visceral leishmaniasis found, making it difficult to implement active case finding in many areas (Mondal et al. 2009).

9.5.3 Addressing Risk Factors Associated with Poverty

Poverty is an important part of the cycle of leishmaniasis. Poor housing contributes to cracked mud walls which provide daytime resting spots for sand flies. Damp earth floors increase the longevity of sand flies, open areas in walls allow sand flies to access people for blood meals and outdoor sleeping contributes to an increased number of sand fly bites (Kesari et al. 2010; Alvar et al. 2006). In addition, malnutrition plays a significant role in worsening disease and the cycle of poverty tends to delay treatment. By working to fill wall cracks, improving flooring, improving nutrition, and increasing education and access to treatment, there are a number of risk factors that can be eliminated (Alvar et al. 2006). Many areas either have no bed nets or have bed nets in poor repair because of lack of funds. In Bihar, the region harboring 50% of leishmaniasis cases, the average family income was under $365 in US currency per year in greater than 75% of those with leishmaniasis (Alvar et al. 2006). To obtain treatment in a non-private facility costs the family nearly $217 in US currency including indirect costs (Meheus et al. 2006). Patients often have to sell assets or seek high interest rate loans to obtain payment for care. Many women and children are not treated because of the high cost to the family (Thakur 2000). As a result, the cycle of poverty is worsened and the risk for acquisition of *Leishmania* continues (Meessen et al. 2003; Bern et al. 2010; Sarnoff et al. 2010).

9.5.4 Zoonotic Control

Zoonotic disease is more difficult to control because of the added complexity of the presence of parasite in animals, people, and sand flies. There are a number of factors contributing to increasing prevalence of zoonotic leishmaniasis. In both the New World and the Old World, migration of people from rural areas to periurban regions has increased disease. The human dwellings have attracted both reservoir hosts and sand flies. By building dams and irrigation systems, the increase in water and crops has increased local reservoir populations, propagating disease. Deforestation, which works to decrease the sylviatic zoonotic cycle tends to change the cycle to peridomestic, domestic, and periurban as reservoirs seek food and shelter near human

dwellings instead of forested regions (Campbell-Lendrum et al. 2001). Human behavior including tourist activity and working in forested areas has put people in closer proximity to the sylviatic zoonotic cycle. There is some suggestion that global warming may extend the sand fly breeding time to allow for overwintering, increasing the time during the year that transmission can occur (Gramiccia and Gradoni 2005; Diniz et al. 2008).

Efforts to prevent zoonotic disease have included forest clearing, destruction of rodent burrows, poisoning rodents, and fogging burrows with insecticide. Prevention of zoonotic visceral leishmaniasis has centered around insecticide treated dog collars, prevention and treatment of canine disease by vaccination, spot insecticide application to dogs, dog-culling, especially used in China and Brazil (Costa, CHN 2011; WHO 2010), and stray dog control. Dog-culling has raised ethical debates and other means have recently been attempted. Mass application of insecticide-releasing dog collars in Italy decreased the incidence of canine leishmaniasis by 86%. In Iran, after one season of dog-collar application, the incidence of human visceral leishmaniasis decreased by 50% (Gramiccia and Gradoni 2005; Alexander and Maroli 2003).

9.5.5 Bed Nets

Bed nets have been shown on an observational basis to decrease the risk of development of kala-azar in Nepal and Bangladesh (Bern et al. 2000, 2005). In the study contradicting these findings, the bed nets were noted to be in damaged repair (Schenkel et al. 2006). Use of noninsecticide bed nets and use of bed nets treated with insecticide has also been shown to significantly decrease the number of human bites in comparison to not using nets in Sudan (Elnaiem et al. 1999). The use of untreated nets decreased the rate of sand fly blood feeding by 85% and decreased human blood intake by 42% (Picado et al. 2009). There are a number of studies documenting a decreased incidence of sand flies in dwellings in visceral leishmaniasis endemic areas with the use of long-lasting insecticidal nets (LLINs) (Picado et al. 2010a; Mondal et al. 2010). Others have indicated only a decrease in the male population with use of LLINs in Bihar (Dinesh et al. 2008). A number of studies have shown a significant decrease in incidence of cutaneous leishmaniasis with use of insecticide-treated nets (ITNs), for example, the incidence of cutaneous leishmaniasis decreased by 97% in Iran with the use of permethrin-treated bed nets (Ostyn et al. 2008). A study was undertaken to evaluate for a similar change in kala-azar incidence with introduction of long-lasting insecticide treated nets in visceral leishmaniasis endemic regions but was unable to show a reduction in seroprevalence with the use of LLINs (Picado et al. 2010b).

9.5.6 Lime Plastering

Lime plastering was undertaken as a means of leishmaniasis prevention when it was recognized that sand fly breeding sites often occurred in crevices in walls and that

holes in walls also allowed for sand fly entry into human dwellings. By using plaster to cover potential breeding sites and sites of entry, there was a marked reduction in sand fly prevalence which persisted for approximately 7 months. In comparison with other means of prevention, lime plastering has similar efficacy but is more costly than other prevention methods, making it less desirable (Joshi et al. 2009).

9.5.7 Vaccination

A number of vaccine approaches have been attempted. As of yet, there are no vaccines in use. Vaccines targeted at prevention using killed *Leishmania* parasites did not show sufficient efficacy in clinical trials. Immunomodulation with vaccines including killed antigen with alum with BCG (Bacillus Calmette-Guerin) (Kamil et al. 2003) have shown improvement in outcome with and without the addition of antimony (Ghalib and Modabber 2007). There are second generation recombinant *Leishmania* antigen vaccines currently being tested clinically and other vaccines including DNA vaccines are being studied in the laboratory (Modabber 2010). It has been proposed that sand fly salivary proteins be used for vaccination given that they illicit a specific immune response and the presence of antibody against salivary proteins has been correlated with protection against visceral leishmaniasis in people (Gomes and Oliverira 2012).

10 Novel Approaches to Vector Control

It is clear that it will take more than current practices to help prevent leishmaniasis globally. Novel approaches to vector control are underway based on the need for improved prevention of vector-borne diseases. Transgenic insects are being made to combat a number of vector-borne diseases. Specifically, mosquitoes have been genetically altered to be incompetent hosts or altered to be released as sterile mating competitors in an attempt to decrease vector numbers in the prevention of malaria and dengue fever (Christodoulou 2011; Ostera and Gostin 2011). There are no published studies to date on the use of this transgenic novel approach on sand flies. Mosquitoes, however, have been transgenically altered in an attempt to prevent leishmaniasis. Using this approach, mosquitoes are transgenically modified to release the sand fly salivary protein SP-15, known to be part of the immunogenic response to *Leishmania* infection, conferring lifelong immunity in those previously infected with *Leishmania* and exposed to sand fly salivary antigen. The transgenic mosquitoes then produce and inject the SP-15 protein when taking a blood meal. The antigen stimulates an immune response which should help prevent development of clinical disease when infected with *Leishmania* (Yamamoto et al. 2010). Another potential novel strategy is the use of *Wolbachia* in sand flies. *Wolbachia* is a bacterium that acts pathogenically in insects such as mosquitoes and sand flies. Using *Wolbachia* to infect mosquitoes in an effort to reduce both

reduce the life span of the insect and make it a less effective pathogen host has been proposed to prevent malaria and dengue. Others have suggested using *Wolbachia* to carry a transgene to alter pathogen transmissibility or to cause vector lethality (Christodoulou 2011; Read and Thomas 2009; McMeniman et al. 2009; Curtis and Sinkins 1998). Recently, *Wolbachia* has been isolated from certain sand fly species, and it has been proposed that a similar approach may be applied to prevention of leishmaniasis by infecting sand flies with *Wolbachia* (Azpurua et al. 2010; Parvizi et al. 2003; Benlarbi and Ready 2003; Cui et al. 1999).

The molecular concept described above is the most studied and promising to date in the prevention of leishmaniasis. It is termed paratransgenesis. Paratransgenesis was initiated for the prevention of other diseases such as Chagas disease, caused by a similar trypanosome parasite. Paratransgenesis takes advantage of genetically manipulating bacteria known to have an association with the vector rather than manipulating the vector itself. In paratransgenesis, there is in vitro genetic manipulation of vector gut bacterial symbionts or colonizers. The bacteria are genetically transformed to enable the export of molecules lethal to the parasite. After manipulation of the bacteria, the bacteria are reintroduced into the gut of the vector where the anti-leishmanial molecules made by the bacteria can effectively render the parasite uninfective prior to transmission of the parasite to the host (Fieck et al. 2010; Durvasula et al. 1997, 1999; Beard et al. 1998, 2001, 2002). In order for this means of prevention to be effective, the following conditions need to be met: (1) there must be nonpathogenic bacteria colonizing sand flies, (2) the bacteria must be amenable to genetic manipulation, (3) the bacteria need to persist a long enough period to effectively halt parasitic transmission, (4) this should occur without significant effect on bacterial fitness, (5) the bacteria must be environmentally friendly in order to safely colonize sand flies with transformed bacteria, (6) the transgene bacterial product needs to interact with the parasite to render the parasite uninfective, and (7) a method needs to exist for dispersal of the transgenic bacteria to an area where sand flies can become colonized without widespread environmental impact.

Studies initiating the work in Chagas disease have shown that the symbiont of the triatomine Chagas vector can be manipulated to release a number of antimicrobial peptides and antibodies having anti-parasitic effects. The project has advanced and will next be progressing to field trials.

Phlebotomus argentipes, the vector responsible for transmission of *L. donovani,* is being studied in a paratransgenic approach to prevention of leishmaniasis. This particular sand fly was chosen as the target vector for a paratransgenic approach because it is the vector responsible for the most cases of the most fatal form of leishmaniasis, with potential to have the largest impact on improving human health and mortality. *P. argentipes* does not have symbiotic bacteria as are found in triatomines (the reduvid vector of Chagas disease), but are populated by a large number of bacterial commensals found in the sand fly gut, most of which are gram-negative enterobacteriacae, thought to arise from the dung-rich environment in which the sand fly breeding sites are found in India. There were additionally gram-positive bacteria identified, including several *Bacillus* species and *Brevibacterium linens* (Hillesland et al. 2008). Both mentioned bacteria are environmentally widespread

and used in applications such as bio-fertilizers and cheese synthesis, both with positive environmental impacts. They are importantly nonpathogenic. Using these bacteria as potential targets for genetic manipulation, *Bacillus pumilus*, *B. subtilis*, *B. megaterium*, and *Brevibacterium linens* were transformed with a plasmid expressing a marker protein. *B. subtilis* was transformed with a plasmid allowing for expression of green fluorescent protein as a marker protein, and it was shown that the bacteria persisted from acquisition during the 4th larval instar stage to the adult sand fly (Hurwitz et al. 2011). Studies have shown a number of antimicrobial peptides (AMPs) lethal to *Leishmania* which can be used as the molecular antiparasitic released by bacteria (Perez-Cordero et al. 2011; Löfgren et al. 2008). Also under investigation are single chain antibodies known to have antiparasitic properties against parasites closely related to *Leishmania* spp. Future steps will involve transforming the bacteria with plasmids allowing for the expression of antiparasitic molecules and studying the efficacy on prevention of *Leishmania* infection. Further detailed studies will also be performed to assess the environmental impact of release of bacteria into the environment. Delivering the transformed environmental bacteria directly to the cow shed breeding sites would allow for an effective means of populating sand flies with the modified bacteria in a novel prevention effort. The use of paratransgenesis is a promising tool that will likely need to be combined with a number of prevention modalities to effectively prevent the continued propagation of this deadly disease.

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Chapter 5
Chagas Disease: Global Epidemiology and Evolving Methods for Control

Nicole Klein, Ivy Hurwitz, and Ravi Durvasula

1 Introduction

Chagas disease, caused by infection with the parasite *Trypanosoma cruzi*, remains a significant cause of morbidity and mortality in Central and South America. Also known as American trypanosomiasis, Chagas disease was discovered in 1909 by Dr. Carlos Chagas and is characterized by chronic cardiac and gastrointestinal manifestations.

In 2002, the World Health Organization estimated 40–120 million people worldwide are at risk for *T. cruzi* infection (WHO Expert Committee 2002). In 2009 the World Health Organization reported 11,000 deaths due to *T. cruzi* infection, and estimated that eight million infected people remain worldwide (Secretariat of World Health Organization 2009). Annual cost of morbidity and mortality attributed to Chagas disease in endemic countries is eight billion US dollars (Schmunis 2000). In 2002, in Latin America, the WHO estimated the burden of Chagas disease to be as high as 2.7 times the combined burden of malaria, schistosomiasis, leishmaniasis, and leprosy (WHO Expert Committee 2002). Though traditionally a disease endemic to Central and South America, due to human migration, there are now significant numbers of people infected with *T. cruzi* in the USA (>300,000), Canada (>5,500), Europe and the Western Pacific (>80,000), Japan (>3,000), and Australia (>1,500). (Schmunis 2007; Schmunis and Yadon 2010) (Fig. 5.1).

T. cruzi infection persists throughout the lifetime of the host, if left untreated. The sheer magnitude of the number of human infections with *T. cruzi*, as well as the significant morbidity and mortality associated with acute and chronic Chagas disease, has sparked extensive interest in approaches to control transmission of the parasite.

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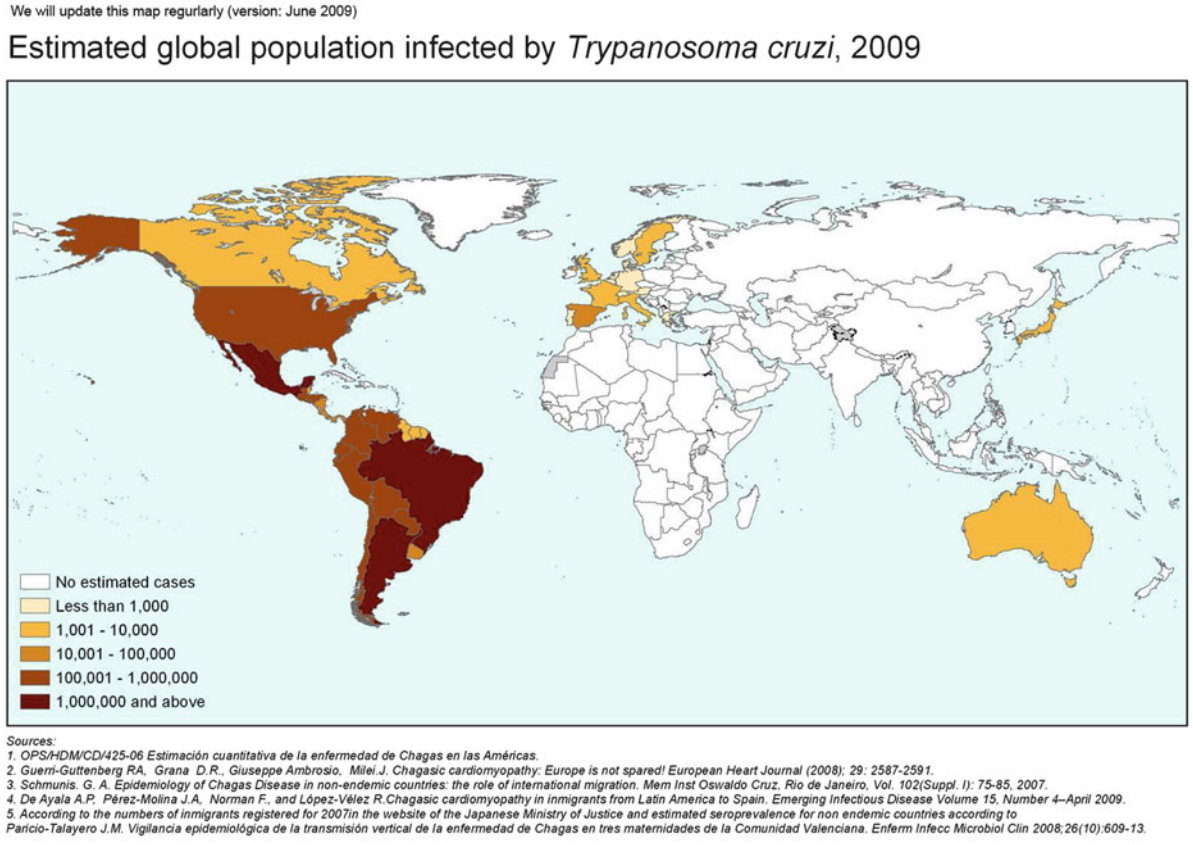

Fig. 5.1 Estimated global population infected by *Trypanosoma cruzi* in 2009 (Anonymous 2009a). Reprinted with permission from DNDI

Human infection with *T. cruzi* most often occurs via a bite from the insect vector of the parasite, the triatomine bug, which lives in traditional human dwellings in endemic countries. The parasite thrives in its habitat from southern Argentina to the southern USA. However, new diagnoses of Chagas disease are increasing in the USA, Australia, Canada, Europe, and Japan (Secretariat of World Health Organization 2009). Because of extensive sylvatic reservoirs of *T. cruzi* in triatomine bugs and wild mammalian hosts, eradication of the parasite in its natural habitat is unlikely. Control efforts to date have focused on large-scale release of pesticides in and around dwellings in endemic areas to prevent infestation with infected triatomines. Though eradication programs have been remarkably successful in some endemic areas, recent surveillance data suggests resurgence of human infections particularly in the Gran Chaco (Gurtler et al. 2007), a large area of lowland plain in South America. In addition, there exist large numbers of people chronically infected with *T. cruzi* who can transmit the infection by blood donation, organ donation, or congenitally from mother to child.

Acute Chagas disease is treatable with appropriate and timely antiparasitic medication. However, chronic disease often goes undiagnosed and can lead to significant cardiac and gastrointestinal disease. Anti-parasite treatment of chronic disease is of questionable clinical benefit. Vaccine development for Chagas disease has thus far been unsuccessful.

Large-scale governmental programs in endemic countries aimed at preventing transmission of the parasite from triatomine bugs to humans through improved housing and education campaigns have been successful to some extent. However, these require extensive time and money to implement and maintain. Therefore, novel approaches to prevent transmission of *T. cruzi* to humans are being developed. These include a molecular genetics approach of paratransgenesis. This technique employs genetic transformation of symbiotic bacteria in the insect host of *T. cruzi*, preventing the carrier state, and thus transmission of infection to humans.

2 History of Chagas Disease

Humans have been infected by *T. cruzi* for thousands of years. Mummies in Chile and Peru, dating as early as 7000 BC to 1500 AD have been noted to manifest chronic changes of Chagas disease. Aufderheide et al. found, in a recent study which employed DNA probe analysis, that 41% of tissue samples from these mummies were positive for *T. cruzi* DNA (Aufderheide et al. 2004). In 1909 a young Brazilian physician, Carlos Chagas, was invited by the Brazilian Central Railroad to a town called Lassance, where immigrant rail workers were dying of what was thought to be malaria (Fig. 5.2). Chagas studied these patients and found they had unique symptoms of cardiac arrhythmias leading to sudden death, not consistent with malaria. He discovered *vinchuca* (triatomine) bugs which were biting them at night

Fig. 5.2 Carlos Chagas (Pinto 2011)

and carried a parasite in their hindgut similar to *Typanosoma brucei*, which causes African sleeping sickness. He named this organism *T. cruzi*, after his mentor Oswaldo Cruz. Chagas went on to elucidate the lifecycle of the organism, including its wild reservoir and vector (*Triatoma geniculata*).

3 Pathogenesis and Lifecycle of *T. cruzi*

Chagas disease is transmitted to humans from mammalian reservoirs in endemic countries by hematophagous arthropod vectors. Triatomine bugs, which belong to the subfamily of Reduviid bugs, become infected with *T. cruzi*, an obligate intracellular protozoan flagellate, upon biting and taking a blood meal from an infected human or vertebrate host. The parasite multiples and matures from epimastigote to metacyclic trypomastigote forms in the midgut and hindgut of the insect. When the triatomine bug takes its next blood meal, it defecates into the bite wound or adjacent conjunctiva or mucous membrane of its victim, excreting metacyclic trypomastigotes in its feces, thereby transmitting the parasite. The parasite enters host cells and multiplies intracellularly by binary fission to form amastigotes. Amastigotes differentiate into trypomastigotes, which eventually cause cell rupture and release into the host bloodstream. Trypomastigotes invade local host tissue, or may travel hematogenously to distant sites to cause infection (Fig. 5.3).

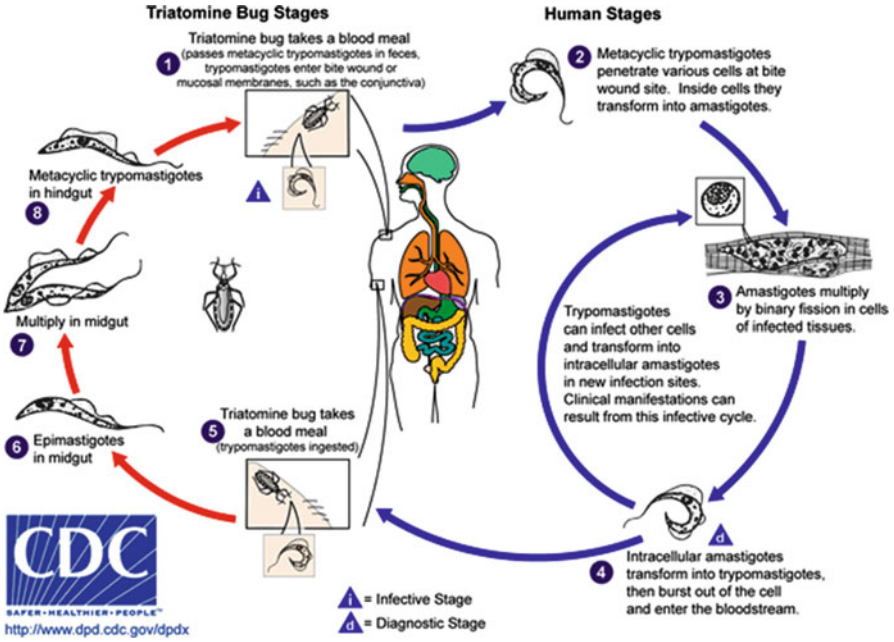

Fig. 5.3 Lifecycle of *Trypanosoma cruzi*. An infected triatomine insect vector (or "kissing" bug) takes a blood meal and releases trypomastigotes in its feces near the site of the bite wound. Trypomastigotes enter the host through the wound or through intact mucosal membranes, such as the conjunctiva (*1*). Common triatomine vector species for trypanosomiasis belong to the genera *Triatoma*, *Rhodinius*, and *Panstrongylus*. Inside the host, the trypomastigotes invade cells near the site of inoculation, where they differentiate into intracellular amastigotes (*2*). The amastigotes multiply by binary fission (*3*) and differentiate into trypomastigotes, and then are released into the circulation as bloodstream trypomastigotes (*4*). Trypomastigotes infect cells from a variety of tissues and transform into intracellular amastigotes in new infection sites. Clinical manifestations can result from this infective cycle. The bloodstream trypomastigotes do not replicate (different from the African trypanosomes). Replication resumes only when the parasites enter another cell or are ingested by another vector. The "kissing" bug becomes infected by feeding on human or animal blood that contains circulating parasites (*5*). The ingested trypomastigotes transform into epimastigotes in the vector's midgut (*6*). The parasites multiply and differentiate in the midgut (*7*) and differentiate into infective metacyclic trypomastigotes in the hindgut (*8*). *T. cruzi* can also be transmitted through blood transfusions, organ transplantation, transplacentally, and in laboratory accidents (Centers for Disease Control and Prevention (CDC) 2011). Reprinted with permission from Nature Publishing Group

4 Triatomine Vectors

Over 150 species of triatomine bugs, also known as *kissing bugs* or *assassin bugs*, exist. However, only those that come in contact with humans are clinically important for transmission of Chagas disease. Triatomine bugs in the wild live in palm trees, burrows, or rock piles and feed, by taking blood meals, on a wide range of vertebrate hosts. Domesticated species colonize human traditional dwellings.

Fig. 5.4 *Triatoma infestans* (Anonymous 2009b)

The genera *Triatoma*, *Rhodnius*, and *Panstrongylus* cause the majority of human infections. In Latin America, *Triatoma infestans* causes two thirds of cases of Chagas disease (Gurtler 2009). *Rhodnius prolixus*, *Triatoma dimidiata*, and *T. brasiliensis* are also known to invade domiciliary structures in Central and South America and transmit *T. cruzi* to humans (World Health Organization 1991). These species, as well as others (including *Rhodnius pallescens*, *Panstrongylus megistus*, and others) comprise the sylvatic reservoir of arthropod-borne *T. cruzi* in Latin America. Recent surveys of reduviid bugs in the American Southwest have shown high rates of *T. cruzi* infection in *Triatoma rubida*, *Triatoma protracta*, *Triatoma sanguisuga*, and *Triatoma gerstaeckeri* (Beard et al. 2003; Dorn et al. 2007; Kjos et al. 2009; Reisenman et al. 2010). However, autochthonous transmission of *T. cruzi* from triatomine bugs to humans in the USA is exceedingly rare, with only seven cases reported. Sylvatic reservoirs also include more than 100 species of mammals; raccoons, opossums, armadillos, foxes, skunks, dogs, wood rats, squirrels, and nonhuman primates (John and Hoppe 1986) being among the most common. Historically, triatomine bugs have subsisted on blood meals from wild mammal species. However, over the last several hundred years deforestation for agricultural and livestock purposes has changed the natural habitat of the bugs. They have adapted over time to live in and around human dwellings, drawing their blood meals from domesticated animals and humans in these areas (Fig. 5.4).

5 Vertebrate Host Reservoirs

Known vertebrate host reservoirs infected with *T. cruzi* in endemic areas include dogs, rats, opossums, guinea pigs, armadillos, wood rats, and raccoons.

6 Modes of Transmission of *T. cruzi*

6.1 Arthropod Vector

Vector-borne autochthonous transmission of Chagas disease occurs by contact with sylvatic or domiciliary infected arthropods. In endemic countries, autochthonous transmission often occurs in childhood, by bite wounds from domiciliary triatomine bugs which live in cracks and thatch of traditional housing structures. Infection by contact with sylvatic bugs outside of domestic areas is also possible.

In addition to vector-borne transmission of Chagas disease, several other mechanisms of human infection are known. These include acquisition of *T. cruzi* parasite by oral or congenital routes, or by blood transfusion, organ transplant, or laboratory accident.

6.2 Oral Transmission

Presumably, contamination of foods or beverages with triatomine feces or ground triatomine bugs or consumption of raw meat of infected sylvatic mammals provides a route for oral ingestion of the parasite. Two outbreaks of orally acquired *T. cruzi* infection associated with sugarcane juice have occurred in Brazil: in Paraiba in 1986 (Shikanai-Yasuda et al. 1991) and in Bahia in 2004 (Benchimol Barbosa 2006; Maguire et al. 1986). In the state of Para, Brazil in 2006, acai juice and acai paste were implicated in an oral outbreak of acute Chagas disease (Nobrega et al. 2009). A large outbreak linked to consumption of contaminated guava juice in school children in Venezuela occurred in 2007 (Alarcon de Noya et al. 2010). Over the last 10 years, approximately 100 new cases per year of orally acquired acute Chagas disease have been reported (Carlier et al. 2002; Dias et al. 2002; Miles et al. 2004) Interestingly, orally acquired acute infection seems to have a longer incubation period of 21 days, compared with 5- to 15-day incubation period of autochthonously acquired infection. Mortality rates for orally acquired acute Chagas disease have been reported to be as high as 28.6% (Dias et al. 2008). In 2009, Benchimol-Barbosa et al. found increasing rates of orally acquired acute Chagas disease between 1982 and 2007 in 5-year cycles correlated with El Nino Southern Oscillation patterns (Benchimol-Barbosa 2010).

6.3 Congenital Transmission

Women infected with *T. cruzi* can vertically transmit the parasite to the fetus, in utero, at a rate of about 5% (Freilij and Altcheh 1995; Gurtler et al. 2003; Torrico et al. 2004). Risk factors for congenital transmission include multiple pregnancies

(Salas et al. 2007), high maternal parasitemia during acute infection, and low immune response to infection in mother or child (Hermann et al. 2004). In 1999, the estimated seroprevalence of antibodies to *T. cruzi* in Latin American pregnant women was 0.4% (Buekens et al. 2008). An estimated 43–54% of congenitally infected infants will be symptomatic (Torrico et al. 2004). In 2004, Torrico found Bolivian mothers infected with *T. cruzi* who transmit the parasite to the fetus have babies with lower APGAR scores, gestational ages, birth weights and lengths, and head circumferences than mothers and babies who are not infected with *T. cruzi* (Torrico et al. 2004). Effect of maternal *T. cruzi* infection on outcome of pregnancy is unclear. A study of pregnant Bolivian women infected with *T. cruzi* showed no effect on gestation outcome, fetal development, or health of neonate when the parasite was not transmitted to the fetus (Torrico et al. 2004). This finding was supported by several studies of maternal *T. cruzi* infection in Brazil (Oliveira Fda et al. 1966; Teruel and Nogueira 1970; Bittencourt 1992). However, there have been two studies in Argentina and Chile, which have suggested increased rates of maternal miscarriage with maternal *T. cruzi* infection (de Castilho and da Silva 1976; Hernandez-Matheson et al. 1983; Schenone et al. 1985.

6.4 Transfusion Related Transmission

In 2009, the World Health Organization reported rates of *T. cruzi* contamination of blood banks in American cities to range from 3% to 53%. In February 2007, the CDC's Morbidity and Mortality Weekly Report described seven cases of transfusion-associated transmission of *T. cruzi* documented in the USA and Canada during the past 20 years. Of note, all occurred in immunosuppressed patients and incubation periods were longer, at 30–40 days (Centers for Disease Control and Prevention (CDC) 2007; Leiby et al. 1999). In 2007, the American Red Cross instituted screening of all blood donors by ELISA for serum and plasma antibodies to *T. cruzi*.

6.5 Organ Transplant Related Transmission

Acute *T. cruzi* infection in solid organ transplant recipients has been reported in Latin America (Centers for Disease Control and Prevention (CDC) 2002). There have also been five reported cases in the USA. In 2001 three organ transplant recipients (37-year-old female who received a kidney and pancreas, 32-year-old female who received a liver, and 69-year-old female who received a kidney) from the same donor who was a Central American immigrant, all contracted acute Chagas disease. All three patients were treated with nifurtimox, one survived and has no evidence of recurrent disease. The other two died—one of acute chagasic myocarditis and the other of unrelated sepsis (Centers for Disease Control and Prevention (CDC) 2002). Two other cases have been reported in the USA. In 2005, a 64-year-old male heart

transplant recipient developed acute *T. cruzi* parasitemia 1 year after transplant. He was treated successfully for *T. cruzi* infection but later died of rejection. Three other organ recipients from the same donor were seronegative by IFA for *T. cruzi*, with no evidence for parasitemia by PCR (Centers for Disease Control and Prevention (CDC) 2006). In 2006, a 73-year-old male heart transplant recipient developed acute *T. cruzi* infection 1 month after transplant. He was treated with nitrofurtimox with resolution of parasitemia, but later died of cardiac failure (Centers for Disease Control and Prevention (CDC) 2006). In another instance, an organ donor, from El Salvador, was RIPA antibody positive for *T. cruzi*. Three other organ recipients from this donor were all negative for *T. cruzi* infection.

6.6 Laboratory Accident Acquired Infection

In October 2001, Herwaldt reviewed laboratory-acquired parasitic infections. She reported 65 laboratory-acquired cases of Chagas disease at that time. Potential routes of exposure in the laboratory include needlestick, wound exposure, transmucosal, vector and possibly aerosol. General duration of the incubation period postexposure is 14 days (Herwaldt 2001).

6.7 Intravenous Drug Use/Sharing Needles

Transmission of Chagas' disease by shared needles in IV drug users with HIV has been documented (Lescure et al. 2010).

7 Clinical Overview of Chagas' Disease

7.1 Acute Chagas Disease

Acute Chagas disease in endemic countries often occurs in children younger than 10 years old and may be asymptomatic or mild and self-limited. Approximately 10–30% of patients with acute *T. cruzi* infection will be symptomatic, with those ages 1–5 years at highest risk (Kirchhoff 2006). The hallmark of acute infection is the chagoma, an inflammatory skin lesion which develops at the site of a triatomine bug bite. The lesion is produced by the parasite which resides intramuscularly at the site of inoculation, attracting a lymphocytic infiltrate, intracellular edema, and adjacent reactive lymphadenopathy. When the triatomine bite is on the face near the eye, the pathognomonic Romana's sign (unilateral palpebral edema, conjunctivitis, and lymphadenopathy) occurs (Fig. 5.5).

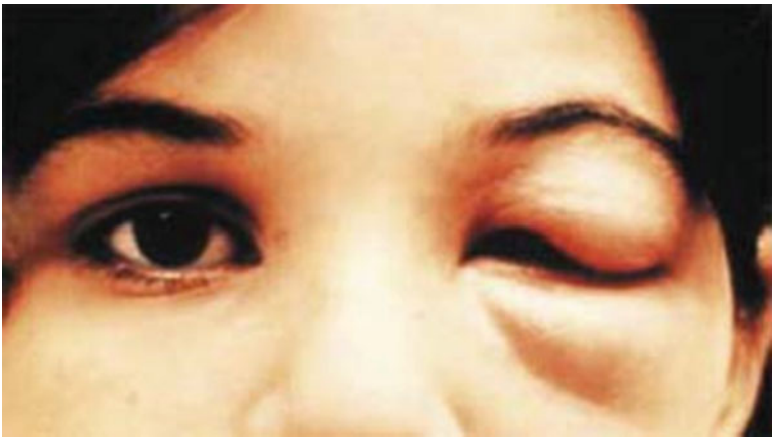

Fig. 5.5 Romana's sign, pathognomonic for Chagas disease, is characterized by unilateral palpebral edema, conjunctivitis, and lymphadenopathy (WHO/TDR 2011)

Nonspecific symptoms of acute infection may also include low-grade fever, malaise, anorexia, general lymphadenopathy, peripheral edema, rash and hepatosplenomegaly. Occasionally acute myocarditis with pericardial effusion and dilated cardiomyopathy or acute meningoencephalitis occurs. Acute myocarditis is manifested by myocyte necrosis, vascular dilation, endocardial involvement with thrombus formation, and conduction system abnormalities. Laboratory abnormalities which often accompany acute infection include lymphocytosis and mild elevation in transaminases. Mortality in acute Chagas disease is about 10%, and is attributed to myocarditis and meningoencephalitis in most cases (Aufderheide et al. 2004). Seventy to ninety percent of infected individuals will remain asymptomatic carries of the parasite (Kirchhoff 2006).

7.2 Chronic Chagas Disease

Survivors of acute Chagas disease progress to an asymptomatic "indeterminate" stage, which can last for decades. During this time, the patient's serologies will be positive, but there is no observable manifestation of disease. Approximately 30% of patients in the indeterminate stage progress to symptomatic chronic Chagas disease, which often manifests in the 4th or 5th decade of life (Maguire et al. 1987; Pinto Dias 1995). Hallmarks of chronic infection include myocarditis, mega-esophagus, and mega-colon. Neurologic, genitourinary, and musculoskeletal manifestations may also be present.

The mechanism of end-organ damage in chronic Chagas disease has long been a controversial issue. It is generally accepted that in acute infection, the host's inflammatory reaction to the parasite itself causes manifestations of disease.

However, whether chronic disease manifestations are a result of host autoimmune response to the parasitic infection, or to ongoing inflammatory response to the parasite itself has been widely debated. Recently, highly sensitive methods of detecting parasite DNA in an infected host have allowed for detection of parasites in infected host tissue in the chronic phase of disease. This suggests that ongoing host–parasite inflammatory interactions may be the mechanisms of chronic disease.

Chronic chagasic myocarditis is marked by chronic cardiac inflammation and fibrosis as well as four-chamber dilated cardiomyopathy, apical aneurysms, intracardiac thrombi, and conduction disturbances. Early conduction disturbances include right bundle branch block and left anterior fascicular block. Late in disease progressive dilatation of the left ventricle with aneurysm formation occurs. In this stage of disease ventricular extra-systoles, nonsustained ventricular tachycardia, sinus node dysfunction, and high degree heart blocks are observed (Hagar and Rahimtoola 1995). Palpitations, presyncope, syncope, and sudden death can all result from chronic Chagasic cardiomyopathy (Rassi et al. 2007).

Gastrointestinal involvement is caused by destruction of parasympathetic and sympathetic neurons in both submucosal and myenteric plexuses of the colon, esophagus or both, leading to mega-esophagus and mega-colon. Patients may have asymptomatic motility disorders or more severe symptoms of achalasia and bowel obstruction (Aufderheide et al. 2004). Dysphagia, odynophagia, gastroesophageal reflux disease, chronic cough with aspiration, abdominal pain, prolonged constipation, and fecaloma are also observed (de Oliveira et al. 1995). Involvement of the stomach is less common, and can result in delayed gastric emptying or gastritis. Chagasic enteropathy involving the small intestine can produce pseudoobstruction or bacterial overgrowth syndrome (Meneghelli 2004). Uncommonly, dilatation of the biliary tree and gallbladder or ureters has been observed.

Interestingly, a geographic pattern of severity of disease has been noted in chronic Chagas disease patients. Fifteen to twenty percent of patients with chronic Chagas disease in the Southern Cone of South America are reported to have chronic gastrointestinal manifestations of disease (mega-esophagus or mega-colon). In contrast, this is rarely seen in northern endemic regions of Central America and Mexico (Kirchhoff 2006). Possible explanations for this observation include variable virulence of regionally specific strains, modulating factors related to regional triatomine populations or differences in host factors.

Neurologic manifestations of Chagas disease may occur in the acute or chronic stages of disease. In acute disease, a broad range of clinical severity can be seen. Mild meningoencephalitis to severe focal paralysis and seizures may occur.

Skeletal muscle myositis characterized by muscle pain and weakness can occur during acute or chronic stages of *T. cruzi* infection. In the 1950s, Cenget and Rojas demonstrated deltoid myositis in 96.3% of a small series of patients with the disease (Cossermelli et al. 1978). This finding was confirmed and associated with presence of parasites in skeletal muscle in the 1970s (Zeledon and Ponce 1972). A case of polymyositis secondary to acute Chagas disease, in a patient with rheumatoid arthritis, has been reported as well (Cossermelli et al. 1978).

7.3 Congenital Chagas Disease

As noted above, 43–54% of congenitally infected infants will have symptoms of infection (Torrico et al. 2004). These may include low birth weight, prematurity, low APGAR scores, hepatosplenomegaly, anemia, thrombocytopenia, petechiae, and anasarca. The most serious manifestations of congenital *T. cruzi* infection include myocarditis, meningoencephalitis, and acute respiratory distress.

7.4 Reactivation of Chagas Disease in the Immunocompromised Host

7.4.1 HIV

Immunosuppressed patients, particularly those with HIV or organ transplant, may present with reactivation of chronic disease. In HIV patients, CD4 count is usually very low, and in many cases less than 50 cells/ml. Reactivation disease in these cases often presents similarly to acute Chagas disease with fevers, rash, and myocarditis. However, CNS manifestations are the most common presenting symptom, including meningoencephalitis and space-occupying lesions including abscesses (which are not seen in immunocompetent patients), seizures, and focal paralysis. CNS lesions may be confused with toxoplasmosis or lymphoma. Parasitemia is often higher in HIV patients with Chagas disease (Sartori et al. 2002). Though mortality in HIV patients with Chagas disease is high, treatment of acute reactivation is effective. A series of three hemophiliacs with both HIV and Chagas disease who were asymptomatic despite high levels of parasitemia has been reported in Brazil (Da-Cruz et al. 2004).

7.4.2 Transplant Patients

Both solid organ transplant and bone marrow transplant can transmit Chagas disease. The immunosuppression that accompanies transplantation can also cause reactivation of disease in a chronically infected host. Diagnosis is made by direct observation of the parasite in the blood, organ biopsy, or compatible clinical syndrome. In organ transplant patients subcutaneous parasite-containing nodules, panniculitis, and myocarditis are common presenting symptoms of reactivation. CNS involvement is less common in these patients (Bern et al. 2007).

8 Diagnosis of Chagas Disease

Several diagnostic tests for infection with *T. cruzi* exist. Screening tests include enzyme-linked immunosorbent assay (ELISA) for antibodies to *T. cruzi* in the serum and plasma, indirect immunoflourescent antibody (IFA), radioimmunoprecipitation assay (RIPA), indirect hemagglutination (IHA), and complement fixation. These

tests have variable levels of sensitivity, but false positive (cross reactivity in autoimmune diseases and other parasitic infections, notably leishmania) and false negative tests are possible. Generally, if one of these tests is positive, a confirmatory test is required for definitive diagnosis. Confirmatory tests include microscopy of blood smear with direct observation of parasites, hemoculture, or PCR for *T. cruzi* DNA. However, in chronic or indeterminate stages of disease, very low levels of parasitemia render these confirmatory tests less sensitive. PCR at this point is only available in the research setting. Overall, serologic testing may be more reliable during indeterminate or chronic stages of disease, when levels of parasitemia are low. However, during acute disease, direct microscopy, PCR, or hemoculture are more sensitive means of diagnosis (Kirchhoff et al. 1996).

An alternative method of diagnosing chronic Chagas disease with low levels of parasitemia is xenodiagnosis. In this method, a triatomine bug is allowed to feed on a patient suspected to have *T. cruzi* infection. The insect is then maintained for weeks in the lab, and then evaluated via fecal samples for microscopic evidence of *T. cruzi*. This technique has reported sensitivity of 69% (Schenone 1999). In 2007, Zulantay et al. showed speed and sensitivity of this method could be increased by using PCR to analyze triatomine bug feces for *T. cruzi* kinetoplast DNA (Zulantay et al. 2007).

9 Overview of Treatment of Acute and Chronic Chagas Disease

To date, treatment of Chagas disease is plagued by medication toxicity, and in some instances, unclear benefit. Nifurtimox and benznidazole are the two antiparasitic drugs recommended for treatment of Chagas disease. Currently, neither is approved for use in the USA by the Food and Drug Administration. However, nifurtimox is available through the Centers for Disease Control and Prevention for compassionate use. Treatment is currently recommended for all cases of acute, congenital, and reactivation disease. Chronic disease in patients younger than age 18 years is recommended as well. Both acute and chronic disease in HIV or organ transplant patients should be treated.

Treatment of chronic Chagas disease with antiparasitic medications is of unclear benefit. Generally, adults aged 19–50 years should be considered for treatment as there is some evidence that progression of disease may be slowed. For patients older than 50 years with chronic disease, treatment is considered optional due to poor tolerability of medications and unclear benefit in clinical outcome (Bern et al. 2007). Treatment with antiparasitic medications has not been shown to alter the course of chagasic cardiomyopathy or chagasic GI manifestations once they are present.

Treatment of acute Chagas disease with either nifurtimox or benznidazole results in cure of parasitemia in approximately 50% of cases (de Andrade et al. 1996; Sosa Estani et al. 1998).

Treatment of Chagasic cardiomyopathy with amiodarone (to prevent arrhythmias), ACE inhibitors (to address heart failure), or implanted cardiac defibrillators (to treat heart failure with low Ejection Fraction+/– arrhythmias) may also improve survival (Rassi et al. 2000, 2001, 2007).

An ongoing trial known as the "benznidazole evaluation for interrupting trypanosomiasis" (BENEFIT trial), is evaluating the benefits of treating *chronic* Chagas disease with long-term benznidazole. One arm of the study employs PCR and serologic testing to monitor changes in parasite burden in patients with chronic Chagasic cardiomyopathy treated with benznidazole. This arm of the study also evaluates the drug's safety profile. A second arm of the study assesses reduction in mortality and progression of Chagasic cardiac disease with benznidazole therapy (Marin-Neto et al. 2009).

Several other classes of drugs are being investigated in treatment of *T. cruzi*. Amiodarone, which is used currently for its anti-arrhythmic effect in Chagasic cardiomyopathy, has also been observed to kill *T. cruzi* in cell culture and animal models. Anecdotal reports suggest that it may reduce levels of parasitemia in infected humans as well (Clayton 2010). Recently, case reports of patients with Chagas disease treated successfully with posaconazole, an antifungal medication, have emerged. A comparative study of posaconazole and benznidazole in murine models which evaluated prevention of Chagasic cardiac disease in 2010 showed suppression of detectable parasitemia in 100% of posaconazole-treated mice at 54 days post-infection versus only 50% suppression of parasitemia in mice treated with benzindazole. In addition, lower levels of cardiac specific enzymes in posaconazole-treated mice suggested less cardiac damage in comparison with those treated with benzindazole (Olivieri et al. 2010). A phase II clinical trial for treatment of Chagas disease with posaconazole in humans is underway. Several drugs which target the cysteine protease of *T. cruzi* are also in development (Doyle et al. 2007).

10 Vaccine Development for Chagas Disease

Toxicity and lack of efficacy of antiparasitic drugs in treatment of chronic Chagas disease has prompted interest in vaccine development. *T. cruzi*'s ability to evade the immunocompetent host's immune response for long periods by hiding intracellularly poses a significant challenge for vaccine development. For this reason, *prevention* of infection with vaccination may be difficult to achieve. However, a vaccine-elicited immune response may be capable of reducing parasite burdens to a level at which host tissue injury and immune dysregulation could be minimized (Cazorla et al. 2008).

Traditionally, concern for worsening of suspected autoimmune components of human response to infection has hampered vaccine development as well. However, some studies now suggest that the persistence of the parasite in infected tissue (and NOT the innate host immune response to the parasite) may actually propagate chronic manifestations of *T. cruzi* infection. For this reason, target antigenic molecules expressed by *T. cruzi* have become the focus of a small body of work on vaccine development.

In the last 5 years, several target molecules expressed by *T. cruzi*, including cruzipain, the trans-sialidase amastigote surface protein and the paraflagellar rod protein

have been the focus of vaccine development. A variety of attenuated viruses and bacteria as well as immunomodulator molecules have been used as DNA delivery systems (Cazorla et al. 2009). Notably, vaccines based on cruzipain antigen, a cysteine proteinase expressed on the surface of the parasite (Souto-Padron et al. 1990), have showed some promising results. Cruzipain is expressed in all developmental stages of the parasite (Parussini et al. 1998), is known to be highly immunogenic (Malchiodi et al. 1994), is involved in parasite internalization into mammalian cells (Parussini et al. 1998), and has been shown to produce a protective immune response (Frank et al. 2003). Because the mode of exposure to the parasite is through skin or mucosal exposure, stimulation of both systemic and local immune responses would be necessary in an effective vaccine. Cazorla et al. (2008) developed a recombinant cruzipain protein and a cruzipain encoding DNA plasmid expressed in attenuated *Salmonella enterica* (serovar Typhimurium). Oral vaccination of murine models produced humoral and cellular immune response, which decreased levels of acute parasitemia in *T. cruzi* challenged mice. In addition, vaccinated mice were examined 100 days post exposure to *T. cruzi* and found to have no evidence of abnormality on microscopic exam of cardiac tissue. Skeletal muscle examination in these mice showed markedly reduced levels of inflammation as well, suggesting that oral vaccination may provide lasting immunoprotection in chronic stages of disease as well.

11 Prognosis of Chagas Disease

The strongest clinical indicator of prognosis in patients with acute or chronic Chagas disease is probability of development of Chagasic heart disease (Bern et al. 2007). Age over 50 years, systolic diameter more than 40 mm, intraventricular conduction disorders, and sustained ventricular tachycardia have been identified as risk factors for progression of cardiac disease (Viotti et al. 2005). Several factors, including virulence of different strains of *T. cruzi* (Andersson et al. 2003), severity of acute infection, age of host at time of infection, and host immune and genetic factors are thought to influence prognosis (Benchimol Barbosa 2006; Bustamante et al. 2003; Campbell et al. 2004; Laucella et al. 2004). A few studies have shown that survival of patients with indeterminate stage Chagas disease is comparable to uninfected individuals (Acquatella et al. 1987; Carrasco et al. 1994; Maguire et al. 1987).

Ventricular conduction abnormalities on EKG predict significant increase in mortality, compared with infected patients with normal EKGs (Maguire et al. 1987). In a retrospective evaluation of 424 Brazilian patients with Chagasic heart disease, Rassi et al. (2006) showed congestive heart failure (NYHA class III or IV), cardiomegaly, LV systolic dysfunction on echocardiography, nonsustained ventricular tachycardia, low QRS voltage, and male sex to be independent predictors of mortality. Based on these findings, a risk score system that estimates the risk of mortality in Chagas patients was developed.

12 Current Concepts in Control of Chagas' Disease

12.1 Background on Chagas Disease Control Strategies

Given the extent of morbidity and mortality attributed to Chagas disease in endemic Central and South American countries, much effort has been aimed toward preventing human infections. These include large-scale governmental campaigns aimed at improved housing (to prevent infestation of sylvatic triatomine hosts in human traditional dwellings) and education, widespread insecticide use, screening blood supplies, prevention of congenital infections with maternal and newborn screening for disease (Fig. 5.6).

Eradication of *T. cruzi* from sylvatic reservoirs in endemic Central and South American countries by application of insecticides is not feasible due to the need for repeated applications over massive areas of native habitat of the triatomine host. Concerns for toxicity of pesticides to other species, including humans, have been a limiting factor. For this reason, application of pesticides to control triatomine populations has been focused on areas of human occupation, to minimize *domiciliary* infestation. Triatomine resistance to pyrethroid pesticides, which are commonly used for this purpose, has been documented in some endemic areas (Germano et al. 2010; Picollo et al. 2005; WHO Expert Committee 2002). In addition, residual action insecticides do not provide permanent protection from reinfestation, and re-spraying is necessary. The cost of widespread pesticide application itself is also a limiting factor. Waxing and waning political interest in this chronic disease has

Fig. 5.6 Endemicity map showing Chagas disease endemic regions of the world (World Health Organization 1991)

plagued funding and sustainability of these programs. Even if eradication of the *T. cruzi* sylvatic reservoir were to be achieved, the large number of chronically infected humans who exist (often undiagnosed) could still transmit the parasite by the maternal–fetal route, or by blood or organ donation.

In 2005 Chagas disease was classified by the WHO as a neglected tropical disease to advocate for control efforts.

12.2 Intergovernmental Initiatives to Control Chagas Disease in Latin America

Successful intergovernmental initiatives between endemic countries in Latin America focused on public education, insecticide use, and improved housing conditions have decreased the annual number of global deaths attributed to Chagas disease from 45,000 in 1990 to 11,000 in 2008 (Guhl et al. 2009; Secretariat of World Health Organization 2009). In addition, the number of chronic worldwide infections has decreased from an estimated 30 million in 1990 to 8 million in 2006 (Secretariat of World Health Organization 2009). Several specific programs and their accomplishments to date are listed here.

1. *Southern Cone Initiative*: An international coalition of governmental agencies from Argentina, Bolivia, Brazil, Chile, Paraguay, and Uruguay formed in 1991 which brought endemic nations together to develop education programs (aimed at reduction of human contact with *T. infestans*) and blood bank screening programs, with the goal of minimizing transmission of *T. cruzi*. Successes of this program include a Pan-American Health Organization awarded certificate for the disruption of Chagas disease transmission by *T. infestans* to Uruguay, Chile, and Brazil (Guhl et al. 2009). Argentina, Paraguay, and Bolivia have achieved partial interruption of vector transmission (Salvatella 2007). However, sustainability of this program has been called into question due to the need for continued application of insecticides and possibility of reinfestation of domestic structures (Guhl et al. 2009).
2. *Initiative of the Andean Countries*: This initiative was formed in Columbia, Ecuador, Peru, and Venezuela in 1997. Venezuela was able to decrease prevalence of Chagas disease in children younger than 10 years old from 20% in 1960 to 0.8% in the late 1990s. However, over the last 10 years seroprevalence seems to be increasing (Aguilar et al. 2007; Ache and Matos 2001).
3. *Initiative of the Countries of Central America*: This was formed in 1997 by the countries of Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, and Panama. Guatemala, Honduras, and El Salvador have achieved elimination of *R. prolixus* (triatomine host) from some areas (Salvatella 2007).
4. *Initiative of the Amazon Countries for Surveillance and Control of Chagas Disease*: This initiative was formed in 2004 and includes Bolivia, Brazil, Colombia, Ecuador, Guyana, Peru, Suriname, and Venezuela (Coura et al. 2002).

Traditionally the Amazon region was not endemic for human Chagas disease. However, in recent years human migration and environmental changes have resulted in more contact with sylvatic triatomine species. *Rhodnius robustus, Rhodnius pictipes, Rhodnius brethesi, Pantrongylus geniculatus*, *and Triatoma maculate* have all adapted to domiciliary life (Guhl et al. 2009).

5. *Mexico National Chagas Programme 2003*: Prevalence of Chagas disease in Mexico is estimated to be 1.5% (Petherick 2010).
6. *WHO Global Network for Chagas Disease Elimination*: This network was established in 2007 to formulate a global approach to Chagas disease, which is emerging in areas outside of Latin America. This prompted development of the nonendemic countries initiative (comprised of Belgium, France, Italy, Spain, Switzerland, United Kingdom, Northern Ireland, Japan, United States), which complements the intergovernmental Latin American initiatives (Secretariat of World Health Organization 2009).
7. *Pan-American Sanitary Bureau Program (PAHO)*: This program was charged with coordinating and overseeing a surveillance system in endemic countries and supporting prevention and control efforts in endemic and nonendemic regions.

13 Concerns Regarding Sustainability of Vector Control Strategies

The lowland plain region of central South America, known as the Gran Chaco, has been the focus of intensive vector control programs aimed to reduce Chagas disease transmission in the last 25 years. In the Argentinian Gran Chaco in particular, an initial campaign of residual pesticide application in 1985 succeeded in reducing domestic infestation with infected *T. infestans* (Gurtler et al. 2007). However, after the initial campaign a resurgence in human infections was observed 2–3 years later (Gurtler et al. 2007; Vazquez-Prokopec et al. 2009). In 2007, Gurtler attributed this failure to several factors. First, the initial pesticide campaign was not followed by adequate and sustained surveillance and control measures. Second, deforestation of this region to support agriculture (soy bean crops) and cattle grazing has led to displacement of rural residents to poor urban areas with substandard housing, and increased sylvatic triatomine infestation of domestic structures (Gurtler 2009). In addition, *T. infestans* developed resistance to pyrethrin and the poor efficacy of pyrethroid insecticides in peridomestic areas (chicken coops, stables) due to frequent exposure to sunlight and high temperatures likely hinders success of this strategy as well (Gurtler 2009). The region has failed to meet the 2005 WHO-established target for interruption of transmission of Chagas disease.

Resurgence of Chagas disease in this region illustrates the innate difficulties faced by vector control programs, creating concern for feasibility, sustainability, and success of vector control programs.

14 Chagas Disease in the USA

Autochthonous transmission of *T. cruzi* is a rare occurrence in the USA. Only five documented cases have been reported to date in the medical literature. However, several studies performed in the southern USA over the last 10 years have documented the presence of *T. cruzi*-infected triatomine bugs and sylvatic mammalian reservoirs. Species-specific behavior of the triatomines found in North America may explain why more autochthonous transmission is not observed. Though autochthonous transmission of Chagas disease in the USA is rare, a significant population of chronically infected humans from endemic countries exists. Through congenital transmission and blood or organ donation, these individuals, who may or may not know they are infected, are able to transmit the infection.

14.1 Mammalian Reservoirs for T. cruzi in the USA

In 2010, Brown et al. (2010) performed direct IFA testing on mammalian hosts to assess seroprevalence of *T. cruzi* among eleven mammalian host species in Arizona, California, Florida, Missouri, Georgia, and Virginia. Seroprevalence of *T. cruzi* antibodies varied by both geographic region and mammalian host. The most common mammalian hosts were raccoons (*Procyon lotor*) with a 0–68% seroprevalence (highest in Missouri) and opossums (*Didelphis virginiana*) with a 17–52% seroprevalence (highest in Florida). In addition, small numbers of striped skunks (*Mephitis mephitis*) in Arizona and Georgia, bobcats (*Lynx rufus*) from Georgia, coyotes (*Canis latrans*) from Georgia and Virginia, and ringtail (*Bassariscus astutus*) from Arizona were seropositive for *T. cruzi*.

14.2 T. cruzi-Infected Triatomine Hosts in the USA

In 2010, Reisenman et al. performed PCR for *T. cruzi* DNA (Reisenman et al. 2010) on gut contents of triatomine bugs from inside and around homes in Tucson, Arizona. Of 164 bugs collected (*T. rubida*, *T. recurva*, *T. protracta*), 41.5% were found to be infected with *T. cruzi*. Of 22 total collection sites, 63% had one or more infected triatomines.

In 2009, Kjos et al. (2009) performed PCR on homogenized gut contents from triatomine bugs collected from 97 counties in Texas. Of 241 bugs collected, 50.74% were positive by PCR for *T. cruzi*. These were found predominantly in the southern half of the state. Of all triatomine bugs tested, 54% of those from domestic settings and dog kennels and 30% of those from sylvatic settings were positive. *T. gerstaeckeri* was the most frequently collected triatomine. *T. sanguisuga* and *Triatoma lecticularia* were also found in domestic settings.

14.3 Autochthonous Cases of Chagas Disease in USA

Five autochthonous cases of Chagas disease have been reported in the USA.

1. 1956 in Greer, Texas.
2. A 56-year-old female from Lake Don Pedro, California was diagnosed with acute Chagas disease by blood smear and culture in 1982. Possible sources of exposure included a squirrel from around her home that tested positive for trypanosomes on blood culture, a dog with a positive complement fixation test, and a triatomine bug with trypanosome positive feces found in a wood rat den around her home. There were six other humans with positive complement fixation titers greater than eight (Navin et al. 1985).
3. A 7-month-old boy died of acute Chagasic myocarditis in Texas in 1996 (Ochs et al. 1996).
4. An 18-month-old child bitten by *T. sanguisuga* in Rutherford County, Tennessee in 1998 was found to have a positive blood PCR for *T. cruzi*, with negative microscopy and hemoculture. Three raccoons in his neighborhood were culture positive for *T. cruzi*. The child was treated successfully with benznidazole (Herwaldt et al. 2000).
5. A 74-year-old female in Louisiana (Dorn et al. 2007) with numerous *T. sanguisuga* bites was IFA antibody positive for *T. cruzi* at a titer of 1:128, and positive by blood culture. She had been asymptomatic of infection. Triatomine bugs from her home were positive by PCR for *T. cruzi* as well.

14.4 Triatomine Behavior and Autochthonous Transmission of T. cruzi in the USA

Variation in triatomine feeding and defecation behaviors is thought to be responsible for low autochthonous transmission of *T. cruzi* in the USA. Klotz et al. (2009) studied feeding and defecation behavior of wild caught triatomines in the American southwest (*T. protracta* and *T. rubida*). Triatomines were fed on live, immobilized mice and the defecation pattern was observed for 1 h. Of the 71 triatomines observed, 30 (42%) produced a fecal droplet within 1 h after feeding. Twenty (67%) of these defecated 1.5–6 cm from the mouse. Eleven (37%) defecated 7–10 cm from the mouse. None defecated on the mouse. In contrast to Central and South American triatomine bugs that transmit *T. cruzi* by biting/feeding on a human host and then defecating immediately into the wound, these data suggest that triatomine species found in the USA may not routinely defecate on the host, thus not routinely transmitting *T. cruzi* autochthonously.

14.5 Blood Bank Screening in USA

In December 2006, the Food and Drug Administration approved an ELISA screening assay, which employs an epimastigote lysate antigen, for detection of *T. cruzi* antibodies in the plasma and serum for use in blood bank screening (Centers for Disease Control and Prevention (CDC) 2007). As of 2007, the American Red Cross screens all blood donors (not all units) for Chagas antibody using this ELISA. A positive screen prompts a confirmatory RIPA. The Red Cross analyzed blood donations from Los Angeles and Oakland, California and Tucson, Arizona between August 2006 and January 2007 (Centers for Disease Control and Prevention (CDC) 2007). In this study, 78.5% of donors agreed to be tested, giving 148,969 donated specimens for testing. Sixty-three specimens (1 in 2365) were repeatedly reactive by ELISA for *T. cruzi* antibodies. Of positive samples by ELISA, 79% were collected in Los Angeles, 14% in Oakland, and 6% in Tucson. All 63 samples positive by repeat ELISA were tested by RIPA. Thirty-one (51%) of these were positive, giving an overall positivity rate of 2.1% in this study.

15 Novel Approaches to Control of Chagas Disease

Due to lack of effective and well-tolerated antiparasitic treatments, and problems plaguing vaccine development, in recent years a novel approach to reducing vector-borne transmission of *T. cruzi*, known as paratransgenic modification, has been developed. This technique entails genetic transformation of bacterial symbionts, which are cohabitants with *T. cruzi* in the gut of the triatomine bug. Symbionts are transformed to express antimicrobial peptides in the gut of the triatomine bug, which kill *T. cruzi*, thus preventing transmission of the parasite to humans.

The technique of paratransgenesis is also under development in our lab and by others for control of other parasitic vector-borne diseases. This includes work with sand fly commensal bacteria to prevent transmission of *Leishmania donovani* (Hillesland et al. 2008; Hurwitz et al. 2011), work with tsetse fly symbionts to prevent transmission of African Sleeping Sickness (Aksoy et al. 2008) and several recent studies with bacterial and fungal commensals of mosquitoes to prevent dengue fever and malaria transmission (Fang et al. 2011; Favia et al. 2007).

15.1 Paratransgenic Modification and Prevention of Transmission of T. cruzi

Paratransgenic modification entails genetic transformation of symbiotic organisms in host vectors to prevent transmission of disease. In Chagas disease, the model insect vector for laboratory studies is *R. prolixus*. This triatomine bug is enterically colonized with *Rhodococcus rhodnii*. *R. rhodnii* is a symbiotic bacterium, which

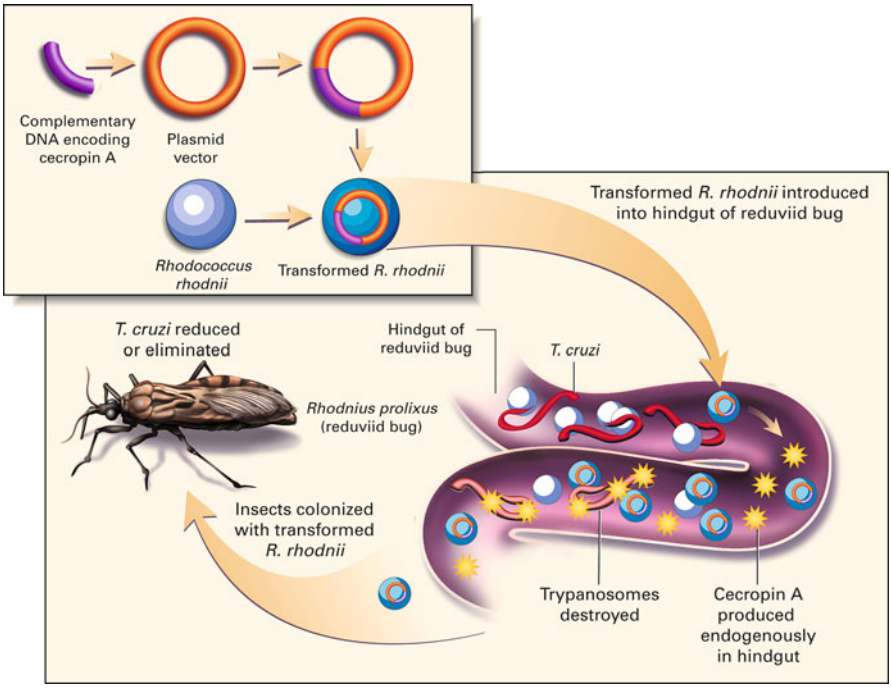

Fig. 5.7 A schematic overview of the paratransgenic approach to control of *Trypanosoma cruzi* transmission by a triatomine bug (Conte 1997). Reprinted with permission from Massachusetts Medical Society

provides *R. prolixus* with essential nutrients not found in the environment. *T. cruzi* lives in the midgut and hindgut of the infected *R. prolixus*, in proximity to *R. rhodnii*. Previous studies have successfully transformed *R. rhodnii* with shuttle plasmids to produce the antimicrobial peptide Cecropin A (Durvasula et al. 1997). Transformed *R. rhodnii* were then introduced into sterile first instar nymphs of *R. prolixus* by coprophagy using synthetic fecal material colonized with transformed *R. rhodnii* (CRUZIGARD) and resulted in elimination of *T. cruzi* infection in 65% of treated bugs (Durvasula et al. 1997), with a 2–3 log reduction in parasite burden in the remaining 35% of treated bugs. Eighty-nine to ninety-six percent of colony forming units (CFUs) of *R. rhodnii* in the paratransgenic bugs exposed to CRUZIGARD preparation were shown to be genetic transformants (see Fig. 5.7).

Ongoing studies have succeeded in transforming *R. rhodnii* to produce other naturally occurring antimicrobial peptides (apidaecin, melittin, magainin). These peptides, produced by transformed *R. rhodnii*, have been tested in in vivo kill assays with *T. cruzi*, and shown to be toxic to the parasite (Fieck et al. 2010).

An additional application of paratransgenic modification for control of *T. cruzi* has been investigated. *R. rhodnii* has been genetically transformed to produce a functional mammalian single chain antibody fragment, rDB3 (Durvasula et al. 1999). *R. prolixus*, a triatomine bug vector of Chagas disease, was then colonized with the transformed *R. rhodnii* via simulated coprophagy using synthetic fecal

material containing the genetic transformant (CRUZIGARD). Production of rDB3 in the gut of *R. prolixus* by transformed *R. rhodnii* was shown to persist for a 6-month period (Durvasula et al. 1999). Furthermore, another triatomine bug vector of Chagas disease, *T. infestans*, was found to have a *Corynebacterial* gut symbiont (Durvasula et al. 2008). This symbiont has also been successfully transformed to produce the mammalian single chain antibody fragment, rDB3, as a precursor to expression of antibodies directed against *T. cruzi*.

Ongoing work with the paratransgenic technique to control Chagas disease entails genetic transformation of *R. rhodnii* to produce combinations of antimicrobial peptides with additive toxicity to *T. cruzi*, and transformation of symbionts to produce lyticase (to break down the outer glycan layer of *T. cruzi* parasite) or alpha manosidase (prevents *T. cruzi* maturation) in the gut of the triatomine bug. Ongoing studies in The Durvasula lab are directed at development of (1) new classes of engineered antibodies that target specific epitopes on the surface of *T. cruzi* (Markiv et al. 2011), (2) recombinant antimicrobial peptides that act synergistically to kill *T. cruzi* (Fieck et al. 2010) and (3) risk assessment methodologies for eventual field application of paratransgenic control (Matthews et al. 2011).

16 Model Development for Paratransgenic Control Strategies

Proof of concept of the paratransgenic strategy for control of vectorial transmission of *T. cruzi* has been achieved under laboratory conditions. Field application of this approach is still a distant prospect and would involve release of foreign genetic material into populations of triatomine bugs via engineered symbiotic bacteria. Therefore, a rigorous and comprehensive risk assessment is mandated prior to consideration of field release. An important part of this assessment involves estimating probability of transgene horizontal transfer (HGT) to environmental organisms. Since HGT plays a vital role in bacterial evolution in many natural settings, the potential for foreign genes to migrate to nontarget bacterial reservoirs poses a risk and should be critically evaluated. Since HGT events are, in general, of low probability, predictive models can play a valuable role as a first step in assessing environmental risk of paratransgenesis. The Durvasula Lab has recently presented a theoretical model predicting HGT in the gut of *R. prolixus* from the genetically transformed symbiont *R. rhodnii* to a closely related nontarget bacterium, *Gordona rubropertinctus*, in the absence of selection pressure. The model treats HGT as a composite event whose probability is determined by the joint probability of three independent events: gene transfer through the modalities of transformation, transduction, and conjugation. Genes are represented in matrices and Monte Carlo method and Markov chain analysis are used to simulate and evaluate environmental conditions. The model is intended as a risk assessment instrument and predicts HGT frequency of less than 1.14×10^{-16} per 100,000 generations (Matthews et al. 2011). Should results of laboratory studies support the predictions of this model, it may be possible to argue that HGT is a negligible consideration in risk assessment of genetically modified *R. rhodnii* released for control of Chagas disease.

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Chapter 6
Integrated Disease Management of Japanese Encephalitis in India

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1 Introduction

Mosquitoes are a well-known group of insects (Order: Diptera, Family: Culicidae) that annoy man and transmit several human diseases. Mosquitoes are the only vectors of pathogens causing malaria, filariasis, Japanese encephalitis (JE), dengue, yellow fever, chikungunya and several other diseases in humans. It was the nineteenth and early twentieth centuries when scientists started to thoroughly investigate transmissible diseases. As early as 1848, Joseph Nott first proposed that yellow fever and malaria were transmitted by mosquitoes. In 1878, Manson showed that the mosquito *Culex quinquefasciatus* transmitted roundworm to humans. Towards the end of the nineteenth century, scientists discovered a number of vector-borne diseases, which were found to be transmitted by arthropods. In 1897, Sir Ronald Ross showed that malaria was transmitted by mosquitoes. Today four species of human malarial parasites, two species of filarial parasites and a number of arboviruses remain as the principal causes of human mortality and morbidity in the world. Approximately 100 of the arboviruses can infect humans, and about 40 are known to infect livestock. Human arboviral diseases are classified clinically by the predominant syndrome caused by them as encephalitis, febrile illness and haemorrhagic fever. Japanese encephalitis virus (JEV), a flavivirus, represents the most significant aetiology of arboviral encephalitis worldwide.

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1.1 Background of Japanese Encephalitis

JE outbreaks were recorded in Japan as early as 1871; the largest, in 1924, involved more than 6,000 cases, 60% of them fatal (Hiroyama 1962). A filterable agent from human brain tissue was isolated in rabbits that year and, in 1934, Hayashi transmitted the disease experimentally to monkeys by the intracerebral inoculation of human brain suspension (Hayashi and Arita 1977; Inada 1937). The virus was subsequently adapted to mice, and soon after, a serological diagnostic test was developed based on the presence of specific neutralizing antibody in recovered patients (Hayashi and Arita 1977; Kawamura et al. 1936a,b; Taniguchi et al. 1936). Inoculation of mouse brain with JE and related St. Louis encephalitis (SLE) flaviviruses provided antigens that enabled workers to confirm encephalitis cases serologically, including a cluster of cases that occurred in 1934 and 1935 in Beijing (Kuttner and T'ung 1936).

The virus was initially called Japanese B encephalitis (the modifying "B" has since fallen into disuse) to distinguish the disease from Von Economo's type A encephalitis, which had different clinical and epidemiologic characteristics. A mosquito-borne transmission for JE was suggested when the virus was isolated from *Culex tritaeniorhynchus* mosquitoes in 1938. Two decades later, field studies established the role of aquatic birds and pigs in the viral enzootic cycle (Buescher and Scherer 1959). Viruses isolated from human cases in Japan in 1935 and in Beijing in 1949 provided prototype Nakayama, Beijing, and P3 strains that were widely used in vaccine production for many years.

In the last three decades, the viral epidemic has switched over to South-East Asian countries (Fig. 6.1) (Umenai et al. 1985; Tiroumourougane et al. 2002; Solomon and Vaughn 2002) and also extended its geographical range to previously unaffected areas of Asia (Cambodia, China, Indonesia, India, Japan, Malaysia, Myanmar, Nepal, Pakistan, Philippines, Republic of Korea, Sri Lanka, Thailand, Vietnam and the South Eastern Russian federation). JE cases were also reported in other non-Asian countries like Torres Strait of Australia mainland (Hanna et al. 1999). Approximately three billion people (roughly 60% of the world's population) live in JE-endemic regions (Halstead and Jacobson 2003). There are estimates of 50,000 cases of JE with 15,000 deaths annually. In addition to the high mortality, about half the survivors have severe neuropsychiatric sequelae (Solomon et al. 1998; Rayamajhi et al. 2006).

1.2 Geography

In endemic areas, the annual incidence of the disease ranges from 10 to 100 per 100,000 population, and 25–30% of encephalitis cases are fatal, with as many as 30% of survivors left with neurological sequelae [Centre for Disease Control (CDC) 2005]. In temperate regions of Asia and the Northern tropical region, JEV is transmitted seasonally. The pattern of JE transmission varies within a country and is also different from year to year (MMWR 1993; Yellow Book: Health Information for

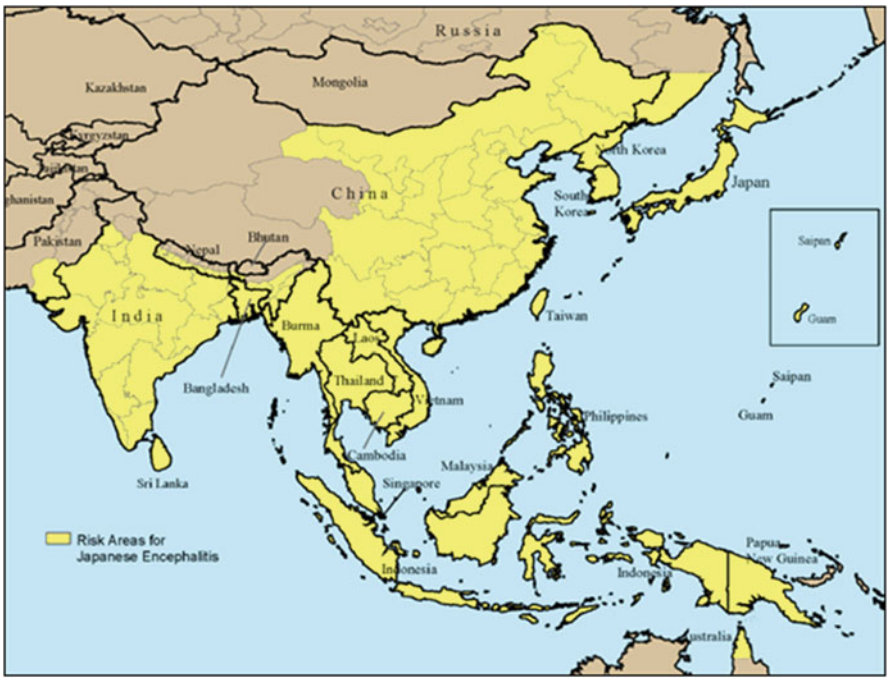


Fig. 6.1 Worldwide distribution of JE

International Travel, 2005–2006, CDC, Atlanta). JE transmission principally occurs in rural agricultural locations where flooding irrigation is mainly practiced. In many areas of Asia, these ecological conditions may occur near or occasionally within urban centres. Transmission is seasonal and occurs mostly during the summer and autumn in the temperate regions of China, Japan, Korea and Eastern Russia. Children and young adults (between 1 and 15 years age groups) are mostly prone to JEV. In recent decades, outbreaks of JE have occurred in several areas, which were previously reported as non-endemic for this disease. The JE outbreak occurs in cycles that may be linked to changing climatic patterns, expansion of irrigated agriculture and pig husbandry (Amerasinghe and Ariyasena 1991).

1.3 JE Outbreaks in India

In India, almost all states have reported JE cases except Jammu & Kashmir and Himachal Pradesh. JE was diagnosed for the first time in India in 1955 at Vellore in North Arcot District, Tamil Nadu (Webb and Pereira 1956). The first major epidemic of JE was reported in the Bankura and Burdwan districts of West Bengal in 1973 where more than 700 cases and 300 deaths occurred; this was followed by the second outbreak that occurred in 1976. Since then, JE outbreaks have been reported

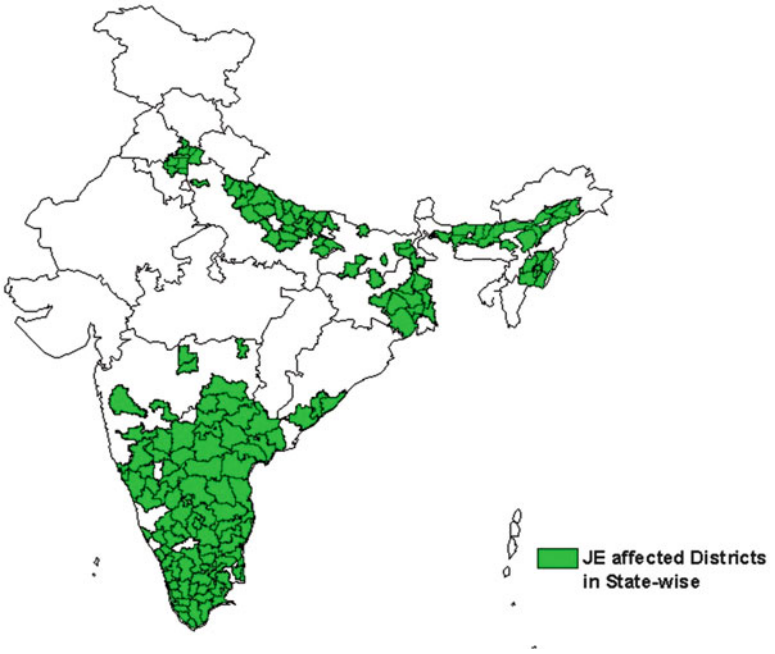


Fig. 6.2 Distribution of JE in India

from these regions, generally during the post-monsoon season. The majority of outbreaks of JE were reported in rural areas (63.63%) and only 36.36% of cases were reported from urban areas of India (Reuben and Gajanana 1997; Roy et al. 2006).

The seasonal incidence of JE varies in different parts of India (Fig. 6.2). The epidemiological studies have shown that after the monsoon rains mosquitoes breed prolifically and carry JEV and subsequently the infection rate of pigs and humans follows (Buescher et al. 1959; Peiris et al. 1992). Many workers have reported the incidence of JE in various states of India during different seasons of a year. Recently, an epidemic of viral encephalitis was reported in Gorakhpur, Uttar Pradesh, India. It was the longest and most severe epidemic in three decades where 5,737 persons were affected in seven districts of Eastern Uttar Pradesh, and 1,344 persons died (WHO 2005).

1.4 Status of JE in Andhra Pradesh, India

The state of Andhra Pradesh (AP) in India has suffered a series of severe epidemics of JE from the late 1970s onwards, and it appears to have become endemic in many districts. JE is one of the most important public health problems in Andhra Pradesh, and the death toll among children due to this disease in this state is reported to be

increasing year after year. Severe epidemics have been reported during 1981, 1986, 1993 and 1999. In 1981, 1273 cases with 439 deaths were recorded. In the subsequent epidemics, the number of cases and deaths had increased considerably as noted in 1986 with 2,048 cases with 640 deaths; as well in 1993 and 1999, more than 1,000 JE cases were reported in AP. The worst affected districts in Andhra Pradesh during 1999 were Kurnool followed by Prakasam, Ananthapur and Cuddapha. The incidence of JE was noticed almost every year in AP in the districts of Ananthapur, Kurnool, Prakasam, Warangal, etc. (Murty et al. 2000). In the year 2000, 343 cases with 72 deaths and in the year 2003, 329 cases with 183 deaths were reported.

2 Aetiology of JEV

JEV is a member of the family *Flaviviridae* which contains more than 60 members of animal viruses (Westaway et al. 1985). By classical serology of cross-neutralization using polyclonal antisera, JEV belongs to one of the eight subgroups or complexes of flaviviruses, such as West Nile, Murray Valley and SLE virus (Porterfield 1980; De Madrid and Porter field 1974; Calisher et al. 1989). JEV consists of a small (50 nm) glycoprotein containing lipid envelope surrounding a nucleocapsid, which encloses one molecule of single-stranded positive sense RNA. The RNA of JEV is approximately 11 Kb long and has positive polarity. This 11 Kb molecule comprises 5^1 and 3^1 un-translated regions (UTRs) between which lies a single open reading frame (ORF) carrying genes for three structural proteins (capsid, premembrane {PrM} and envelope {E}) and seven non-structural (NS) proteins (Svitkin et al. 1981; Monckton and Westaway 1982; Smith and Wright 1985). The JEV genome contains 10,976 nucleotides and encodes a single ORF of 10,296 nucleotides corresponding to 3,432 amino acid residues (Sumiyoshi et al. 1987).

2.1 Life Cycle of JEV

JEV is a mosquito-borne zoonotic flavivirus that infects vertebrate hosts in an enzootic cycle primarily in birds and swine (Vaughn and Hoke 1992). JE maintains a complex life cycle that involves pigs as amplifying hosts, ardeid birds as reservoirs and mosquitoes as vectors (Fig. 6.3). The *Culex vishnui* subgroups of mosquitoes are the major vectors and play an important role for JE epidemiological outbreaks in India (Mishra 1984). The other species of *C. vishnui* subgroup include *C. tritaeniorhynchus Giles*, *C. vishnui Theobald* and *C. pseudovishnui Colless*. These species are extremely common, widespread and breed mainly in paddy fields, sunlit pools, roadside ditches, tidal marshes of low salinity or man-made containers (Mogi 1984; Sucharit et al. 1989). *C. tritaeniorhynchus Giles* is the principal vector throughout East, South-East and South Asian countries. JEV has been isolated from other mosquito species such as *Anopheles* and *Mansonia* mosquitoes (Indian Council of Medical Research (ICMR) Bulletin 2000). JE vectors are predominantly

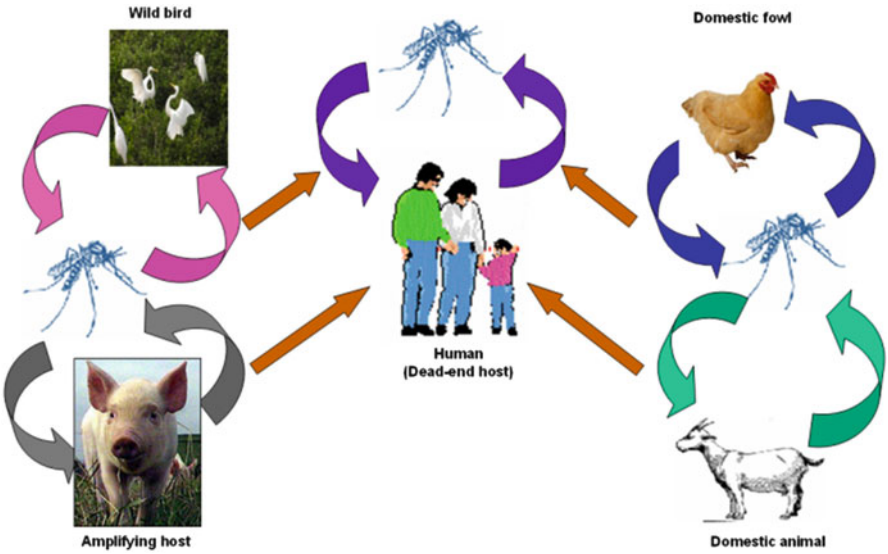


Fig. 6.3 Life cycle of Japanese encephalitis

pig and cattle blood feeders and humans are the dead-end hosts (Self et al. 1973). Humans become infected inadvertently when they encroach on this cycle, but they are considered "dead-end" hosts because normally they do not have sufficiently high or prolonged viraemia to transmit the virus further (Solomon 2004).

After the bite of an infected mosquito, the virus is thought to amplify peripherally, causing a transient viraemia before invading the central nervous system (CNS) in the host. The animal models reveal that the site of peripheral amplification is thought to be dermal tissues and lymph nodes. In experimental studies with a hamster model of SLE virus (a related flavivirus), the olfactory route was shown to play an important role in the transmission of virus to the CNS in the host (Monath et al. 1983). However, immunohistochemical staining of human postmortem material has shown diffuse infection throughout the brain, indicating a haematogenous route of entry (Desai et al. 1995; Johnson et al. 1985). Although experimental evidence suggested that replication within endothelial cells may be an important means of crossing the blood–brain barrier in some flaviviruses, for JEV passive transfer across the endothelial cells seems a more likely mechanism (Dropulie and Masters 1990; Liou and Hsu 1998).

2.2 Transmission of JE

Human transplacental transmission was observed for the first time during an epidemic of JE in Uttar Pradesh during 1978 (Chaturvedi et al. 1980; Mathur et al. 1982).

JEV has been implicated as causing intra-uterine infection in swine with stillbirths and the virus recovered from the brain of stillborn piglets (Morimoto et al. 1972). JEV transplacental transmission and foetal injury has also been observed in humans (Chaturvedi et al. 1980) and experimentally in mice (Mathur et al. 1981). JEV infection can be transmitted to the foetus during consecutive pregnancies. JEV is also maintained in nature in vector mosquitoes by transovarial transmission also known as vertical transmission (Arunachalam et al. 2002; Thenmozhi et al. 2001; Soman et al. 1986).

2.3 Clinical Symptoms of JE

Humans become infected with JEV coincidently when living or traveling in close proximity to the enzootic cycle of the virus. The incubation period for JEV is 9–12 days in man and 5–15 days in mosquito. The clinical symptoms progress through four stages:

1. *Prodromal illness (2–3 days)*—Onset may be characterized by abrupt headache, respiratory symptoms, anorexia, nausea, abdominal pain, vomiting and sensory changes, including psychotic episodes. A low-grade fever or minor respiratory symptoms may be the only clinical expression of JE.
2. *Acute stage (3–4 days)*—It is heralded by high fever, convulsions, confusion, disorientation, delirium or somnolence progressing to coma. There may be occurrence of oliguria, diarrhoea and relative bradycardia. Fatal cases usually progress rapidly to coma and the patient dies within 10 days.
3. *Sub-acute stage (7–10 days)*—The severity of the CNS disease lessens, but pneumonia, urinary tract infections or bedsores may be management problems and in some instances are life threatening.
4. *Convalescence stage (4–7 weeks)*—It is prolonged with weakness, lethargy, incoordination, tremors and neuroses and weight loss observed.

Neuropsychiatric sequelae are reported in 50% of survivors and miscarriages have been observed in pregnant women. Pathological changes such as hyperplasia of germinal centres of lymph nodes, enlargement of malpighian bodies, interstitial myocarditis, hyaline changes in hepatic Kupffer's cells, pulmonary interalveolitis and focal haemorrhages in the kidney are also observed due to JE infection (Tiroumourougane et al. 2002). Similarly, seizures, Parkinsonian feature, flaccid paralysis, excess salivation, or irregular respiration and movement disorders are common both in the acute and sub-acute stages of infection and as part of the sequelae (Solomon et al. 1998, 2000; Misra and Kalita 2002). Asymptomatic JEV infection raises IgM in serum, without antibody production in the CSF (Innis et al. 1989). In clinically significant cases anti-JEV IgM in CSF is elevated, indicating active antibody production in the CNS in response to viral invasion (Burke et al. 1982).

2.4 Population at Risk for JE

According to recent reports, it is estimated that two billion (including 700 million children) people are at risk for JE. Around 1.9 billion people currently live in rural JE-prone areas of the world and the majority of them are found in China (766 million) and India (646 million). According to WHO (2004), the global burden of JE was 709,000 disability adjusted life years (DALYs) and annual incidence was 175,000. It was estimated that nearly 1,025,000–1,080,000 km^2 of land is irrigated in JE-prone areas, and currently 180–220 million people are living close to irrigation or rice-irrigation schemes in the JE-endemic regions. About 90% of the world's rice is produced in Asia and, where JE outbreaks frequently have been reported, it is noticed that rice cropping is the main source of JE infection (Consultative Group of International Agricultural Research 1998).

3 Agriculture Impact on JE

JE vector abundance is closely related to agro-climatic features, generally temperature, and monthly rainfall. The most important causative factor of JE vectors is the water level in paddy fields. The physical and chemical properties of rice field water exhibit marked variations during the day and during the crop cycle (Roger and Kurihara 1988). The traditional method of rice cultivation is to maintain a depth of 5–15 cm water throughout the period of the rice crop except for a period of 10–14 days when the field is allowed to dry out prior to harvest (ICMR Bulletin 1992). In Thailand, the highest number of larvae and pupae of JE vectors were collected when the rice fields were ploughed with water in the fields. The vector population decreased after transplanting when the fields were flooded, and stayed low until harvest (Somboon et al. 1989). The height of the rice plant, water, temperature, dissolved oxygen, ammonia nitrogen and nitrate nitrogen, pH, ionic composition and conductivity are reported to influence larval density and their rate of development (Kramer and Garcia 1989; Sunish and Reuben 2001). In rice fields of Japan there was an increase in the numbers of *C. tritaeniorhynchus* larvae at the time of transplanting, when the water became suitable for mosquito breeding as a result of fertilizer application (Wada 1974). Urea fertilizer was found to act as an oviposition stimulant for *C. tritaeniorhynchus*, as 91.4% of egg rafts of this species were collected from urea treated quadrants versus untreated quadrants (Sunish et al. 2003). Immature mosquito populations declined with the growth of the paddy stands. This decline could be due to a decrease in the water temperature and sunlight, and a resultant decline in the growth of microorganisms upon which the mosquito larvae depend (Ramachandra Rao 1984). In the years 1985–1986 and 1987–1988, an epidemic of JE occurred in Mahaweli System H, Sri Lanka. This outbreak occurred due to high irrigation and pig husbandry, while no cases were reported from non-irrigated areas with few pigs (Amerasinghe 2003).

In India, a high incidence of JE vectors has been noticed in Gorakhpur district of Uttar Pradesh and Mandya district of Karnataka, well-grounded through the paddy cultivation and irrigation practices followed in these areas (Mishra 1984; Kanojia et al. 2003). In Assam, 78.6% of the JE cases occur due to the practice of paddy cultivation (Phukan et al. 2004). An estimated 378 million population are living at the risk of JE in 12 States and Union Territories of India, which are frequently affected. The spread of JE to new areas is probably due to agricultural development and intensive rice cultivation supported by irrigation schemes (Rao 2000).

3.1 Impact of Climate on JE

Climate is a major component in the environment of all arthropods, and indeed all living organisms. All species live within defined climatic limits, although the actual limits of their distribution are only partly determined by climate. Weather also exerts a profound effect on arthropods. Within their climatic limits, all the atmospheric elements constantly affect every aspect of behaviour, development and dispersal, while at the boundaries of their climatic limits, relatively minor deviations from the ambient norm can be catastrophic.

3.1.1 Adaptation of Vectors

In the tropics, many insects restrict their active phases to a portion of the wet or the dry season, or both (Denlinger 1986). In addition to these preprogrammed periods of inactivity, transient events, such as periods of low temperature or heavy rainfall may interrupt normal feeding and reproductive behaviour during an active season, or may interrupt or terminate inactivity during a dormant season (Beck 1983). In most species, programmed dormancy is expressed in one stage of the life cycle. For example, *Culex* and *Anopheles* species survive as adults in winter or drought seasons. The alternative to seasonal dormancy is to continue reproduction throughout the year, with opportunistic bursts of population increase whenever conditions are suitable. In these circumstances, climatic seasonality dictates abundance by the availability of food or breeding sites. A notable example is dormancy in *C. quinquefasciatus*; in the tropics this species breeds throughout the year, yet there is evidence (Gillett 1971) that during the dry season a large fraction of the adult population becomes inactive. A problem with this type of dormancy is that when all stages of the life cycle occur simultaneously, its presence may be difficult to demonstrate.

3.1.2 Vagaries of Climate

JE causes severe epidemics which are highly seasonal, occurring during the monsoon season when temperatures reach 30°C or above (Mellor and Leake 2000). Rao

(2000) observed that JE cases peaked with an increase in temperature and rainfall in India. These vagaries of climate are considered as highly elusive risk factors of JE. The seasonal patterns of this viral transmission are correlated with the abundance of vector mosquitoes and of vertebrate amplifying hosts. The abundance of vector mosquitoes fluctuates with the amount of rainfall and with the impact of the rainy season, especially in tropical situations, though agricultural practices are considered a more important factor affecting vector abundance (MMWR 1993).

3.1.3 Overwintering

Several theories have been put forward to explain the persistence of the virus from one epidemic season to the next. The virus may overwinter in hibernating mosquitoes, in mosquito eggs, in reptiles, or it may be reintroduced by migrating birds. Some investigators have proposed that the virus remains in hibernating mosquitoes or is transmitted to their offspring. In Korea, a collection of 50,499 mosquitoes during the winter months over a 6-year period revealed two strains of JEV (one in December and one in February) (Lee 1971). JEV has also been isolated from larvae collected in June (1 of 382,000 examined) suggesting vertical transmission of the virus in *C. tritaeniorhynchus* mosquitoes as a possible inter-epidemic viral survival mechanism (Rosen et al. 1989). Maintenance of virus in hibernating mosquitoes may be the principal method of overwintering (Rosen 1986). The virus may be transmitted year round in tropical climates and reintroduced into temperate climates by migrating birds or by mosquitoes that are blown by the wind or carried in vehicles (Lee 1971).

There is clearly a wealth of evidence that weather plays a role in arboviral recrudescence. Behind most attempts to rationalize this role is the hope that weather forecasting and weather analysis might eliminate the element of surprise in arboviral epidemics. But the essential fact is that arboviral epidemiology is a complex, multifactorial process, and those coincidental events involving some or all variables are the true precipitating factors for recrudescence.

3.2 Socioeconomic Status

JE transmission closely correlates with the socioeconomic status of a population. In central China, more JE cases were observed among children living in poor quality houses and whose parents have low income (Luo et al. 1995). In poorer regions, exposure to domestic animals, household crowding, low socioeconomic status and lack of proper ventilation appear to be the risk factors for acquiring JE (Aaskov et al. 1993; Halstead and Jacobson 2003). To raise the economic standards, the poorer section or low socioeconomic status of the community has accepted pig and mini-poultry farming as accelerated sources of income. These animals are known to be the favoured reservoirs of *flavivirus* infection. The majority of people in poor

rural areas live under the same roof with their animals, which brings the animals into close contact with humans, and thus the community becomes exposed to JE infection through mosquitoes (Chatterjee et al. 2004).

4 Host Preference of JE Vectors

The increase in the number of host contacts with humans as a result of multiple feeding may increase disproportionately the rate of encephalitis virus transmission by *Culex tarsalis* (Anderson and Brust 1995). Thus host–vector contact is an important parameter in JE epidemiology. Multiple feeding was reported in field populations of vectors of malaria, Eastern equine encephalitis, SLE and Western equine encephalitis. The host preference of mosquito vectors may be influenced by a number of factors including host availability and genetically determined factors. The important JE vectors in Asian and South-East Asian countries, *C. tritaeniorhynchus*, *Culex gelidus* and *C. vishnui*, have shown to feed mainly on cows in some places and pigs in other places depending on host availability (Reuben and Gajanana 1997). When the host preference of JE vectors was examined in Thailand with equal availability of hosts, they were shown unequivocally to prefer cows to pigs (Mwandawiro et al. 1999). It has been suggested that genetic variability may also affect the behaviour of mosquitoes. It has also been reported that genetic heterogeneity influences the feeding preferences of *Aedes aegypti* and *Aedes simpsoni* (Mukwaya 1977) and the house-entering behaviour of *A. aegypti* (Trpis and Hausermann 1978).

4.1 Feeding Patterns

The JE vectors *C. tritaeniorhynchus*, *C. gelidus* and *C. vishnui* exhibited a tendency to feed preferentially on cows or pigs, depending on which host they had been previously attracted to, or had previously fed upon. Host availability can affect the range of hosts to which a particular mosquito species orientates in nature and can produce a feeding pattern determined by repeated contact with a particular host rather than a fixed feeding behaviour (Edman et al. 1972). According to Mwandawiro et al. (2000), the JE vector species feed more on cows than on pigs. Therefore, an increase in the availability of cattle would affect the spread of JE by diverting mosquito vectors from pigs to cows due to increased probability of mosquitoes biting cattle early in their life.

In India, the multiple feeding pattern of JE was first observed in JE-endemic areas of Kerala. A field population of *C. tritaeniorhynchus* associated with JEV transmission was evaluated for multiple feeding to determine the frequency of contact with more than one host in a gonotrophic cycle. The frequency of multiple feeding on cattle and goats is very high in *C. tritaeniorhynchus*. It may be due to the

availability of the animals under the same roof. However, it is clearly evident that JE vectors prefer to feed on cattle (Arunachalam et al. 2005). This multiple feeding on various hosts may favour virus transmission if the vector feeds on all potential hosts or reduce transmission if the vector feeds on unimportant hosts that are not implicated in human transmission. In Northern Australia it was observed that the vectors showed high rates of pig feeding. More populations of domestic pigs were housed in piggeries where the density or availability of pigs is a major factor that influenced the feeding patterns of *Culex annulirostris* in these areas. In Badu Island, 96% of *C. annulirostris* obtain their blood meal from pigs (Van den Hurk et al. 2001). Host preference experiments by Kay et al. (1979) at Kowanyama demonstrated that *C. annulirostris* preferentially fed on pigs when compared to marsupials.

There are a number of factors, either environmental or genetic, that can potentially influence the host-feeding patterns of mosquitoes (Washino and Tempelis 1983; Edman and Spielman 1988). Environmental determinants of mosquito host-feeding behaviour include weather variables such as temperature, photoperiod, wind speed and direction, as well as vertebrate host density, availability and host behaviour (Tempelis 1975).

5 Prevention of JE

JEV (family *Flaviviridae*, genus *Flavivirus*, JEV) is currently one of the most important arboviral childhood viral encephalitides in Asia. Because of a poor surveillance system in India, the actual burden of JE cannot be estimated. The actual disease burden could be estimated only by strengthening diagnostic facilities for JE confirmation in hospitals. However the available records at present indicate that there is a rising trend in JE occurrence and expansion of the disease into non-endemic areas. JE control through vector control methods has limitations owing to sustainability and cost-effectiveness of the programs. Under these circumstances, the feasibility of JE vaccination in India has to be regarded as a preventive measure, for which identification of risk areas, target population to be immunized and cost evaluation of immunization is to be emphasized. Since JE vaccine is produced in India, extension of the availability of this vaccine into routine JE immunization programs is not a remote prospect (Kabilan 2004).

There has been a reduction of JE cases in developed countries, such as Japan and Korea, due to several factors. The secular trends toward a higher standard of living, reduction in farming, changes in agriculture practices (increased use of pesticides), centralized pig production and immunization of humans and pigs against JE contributed towards the decline of JE cases. However, these changes have been accompanied by a shift in the age distribution of cases toward older children and to adults and have been attributed to waning immunity (Lowry et al. 1995; Ayukawa et al. 2002). In India, vast secular changes are progressing in urban areas, which are yet to occupy rural areas; thus, there has not yet been a shift in the age distribution of

JE cases. The benefits of vector-borne disease control programs were short-lived. A number of vector-borne diseases began to re-emerge in the 1970s, a resurgence that has greatly intensified in the past 20 years (Gubler 1996; Krogstad 1996; Bruce-Chwatt 1979; Hammon 1973). Although the reasons for the failure of these programs are complex and not well understood, two factors played important roles: (1) the diversion of financial support and subsequent loss of public health infrastructure and (2) reliance on quick fix solutions such as insecticides and drugs.

5.1 Surveillance

The component of JE surveillance consists of three major areas:

1. Clinical/syndromic surveillance through the Primary Health Centre (PHCs) system for early diagnosis and proper management of JE patients.
2. Vector surveillance in JE-prone areas for monitoring vector behaviour and population build-up for timely implementation of intervention methods.
3. Sero-surveillance to delineate high-risk population groups and to monitor JE specific antibodies in sentinel animals or birds as an indication of increasing viral activity.

5.2 Vector Control

The extensive and intensive rice cultivation is responsible for epidemics of JE, which occur mostly after a few years of drought followed by heavy rain. This causes a sudden burgeoning of the vector population and subsequent disease transmission (Rajagopalan and Work 1996). Environmental pollution due to insecticide spraying and development of resistance by mosquitoes against an array of insecticides discouraged the use of insecticides in vector control programs. *C. tritaeniorhynchus* is highly resistant to DDT, dieldrin (Lee 1969), Malathion, propoxur (carbamate) and permethrin (pyrethroid). Because *C. tritaeniorhynchus* breeds mostly in irrigated paddy fields, it would have been exposed to pesticide selection pressure (Karunaratne and Hemingway 2000).

Biopesticides like *Bacillus sphaericus* and *Bacillus thuringiensis var israelensis* were promoted and anticipated to have great potential as biological larvicides against different mosquito species. A single application in a rice field of a microgel droplet of *B. sphaericus* reduced the density of immature culicine vectors of JE (Sundararaj and Reuban 1991). Lack of a suitable delivery system, development of resistance to this biopesticide by mosquitoes and short duration of larvicidal effect restricted its use in vector control strategies.

5.2.1 Indoor Residual Spray

Vector control is a serious challenge for JE control because of exophilic and exophagic behaviour of JE vectors, which limits effectiveness of conventional vector control methods like indoor residual spray (IRS). Hence IRS is not recommended for prevention and control of JE. However, in areas where the vector is endophilic, like *Mansonia annulifera*, IRS may be considered for vector control in high-risk pockets.

5.2.2 Reduction in Man–Vector Contact

Commercial mosquito repellants in the form of coil, cream and mats are widely deployed to repel mosquitoes. Pyrethroid-impregnated bed nets and curtains have been shown to reduce man–mosquito contact. Impregnated bed nets, a low cost technology, were implemented on a pilot scale in JE-endemic areas of Cuddalore district of Tamil Nadu. Pyrethroid-impregnated jute/polypropylene curtains were used for protection against endophagic mosquitoes. The curtains were found to be effective and have proven efficacy in the control of JE vectors (Tsai 1990). The limitation with this technology is the need for repeated impregnation of the curtain every 6–9 months and periodic assessment of vectors for development of insecticide resistance.

5.2.3 Fogging

Fogging is a very cost-intensive vector control tool with limited effect and therefore is not recommended as a routine vector control measure. In the case of JE outbreaks, since the vectors are mainly outdoor resting and outdoor feeding, peri-domestic fogging could be attempted very carefully for containment of outbreaks. It has been suggested that most parts of India may resort to fogging whenever there is a JE outbreak, so that they can make their efforts visible in the community and exert some impact on adult population of vector mosquitoes.

5.2.4 Reduction of Breeding Sources for Larvae

Two feasible methodologies have been demonstrated to control breeding of mosquitoes in rice fields. They are:

1. Water management systems with intermittent irrigation devices which conserve water resources, reduce vector abundance and increase rice yields.
2. Incorporation of neem products in the soil to promote better utilization of urea by killing nitrifying bacteria in the soil while simultaneously reducing vector breeding.

The water management is a strategy of alternate drying and wetting water management practices in rice fields. This process could significantly reduce, but not eliminate, vector breeding. Use of neem products as fertilizer in rice fields not only enhances grain production but also suppresses the breeding of culicine vectors of JE (ICMR Bulletin 1992).

5.2.5 Control of Pigs

Pigs promote amplification of JEV. When mosquitoes bite the pig, the animal gets infected and serves as a reservoir for transmission to humans. In JE-endemic areas, pigs are found associated with human habitations. Control methods can include immunizing or slaughtering of pigs. Segregating pigs at least 4–5 km away from human habitations can be used wherever possible by implementing by-laws through local administrations. Several studies conducted in Japan showed that pig immunization was effective in eliminating the disease in pigs, which may reduce the amplification of virus in the animal and possibly trigger lower rate of transmission to humans. This has not been used at the national level because pig immunization requires large numbers of newborn pigs to be immunized each year and the effectiveness of vaccine is for a limited period only.

5.2.6 Immunization Against JE

There are three types of JE vaccine in production worldwide for control of JE. These are (1) inactivated mouse brain-derived vaccine; (2) inactivated primary hamster kidney cell-derived vaccine; and (3) live attenuated vaccine.

JE vaccine used in India is a formalin-inactivated product prepared from mouse brains infected with Nakayama JEV manufactured at Central Research Institute, Kasauli, Himachal Pradesh. JE is one of a short list of vaccine-preventable diseases, and vaccination against JE ideally should be routinely practiced in all areas of Asia where the virus is responsible for human disease (Monath 2002). The mouse brain-inactivated vaccine using the Nakayama strain has been in use for several decades in Japan (Banerjee et al. 1988). Countries like Japan, Taiwan and South Korea, China and the Democratic People's Republic of Korea routinely vaccinate all school-age children against JE (Monath 2002). Limited studies in South Arcot district, which is endemic for JE in Tamilnadu, have shown that the vaccine is reasonably effective in boosting the neutralizing antibody response (Banerjee et al. 1988).

However, the problems associated with vaccine for JE are different and will have to be addressed at different levels. Firstly, there is a great discrepancy in age at which vaccine should be administered. In endemic areas, children are affected whereas adults are frequently more affected in new areas of transmission. The high cost of currently available vaccine and the additional cost of administering three doses will also have to be taken into consideration. There is a need for a proper pilot trial on JE vaccination involving 3–4 JE hyper-endemic districts before implementing

a policy on mass JE vaccination in the community. Vaccination of young children under the age of 15 years may be taken up in the worst affected areas. For immunoprophylaxis against JE, two doses of vaccine followed by a booster dose are to be administered during the inter-epidemic period, at least 1 month before the onset of transmission season (Banerjee et al. 1988).

5.3 Factors Involved in Vector-Borne Disease Emergence

The factors responsible for the emergence/resurgence of vector-borne diseases are complex. They include insecticide and drug resistance, changes in public health policy, emphasis on emergency response, de-emphasis of prevention programs, demographic and societal changes, and genetic changes in pathogens (Lederberg et al. 1992). Major global, demographic and societal changes of the past 50 years have directly affected the emergence of vector-borne and other infectious diseases (Lederberg et al. 1992; Gubler 1997). Unprecedented population growth, mostly in developing countries, resulted in major movements of people, primarily to urban centres. This unplanned and uncontrolled urbanization (inadequate housing, deteriorating water, sewage and waste management systems) produced ideal conditions for increased transmission of mosquito-borne, rodent-borne and water-borne diseases. Other societal changes, such as agricultural practices and deforestation (Lederberg et al. 1992), increased the risk for vector-borne disease transmission. Many irrigation systems and dams have been built in the past 50 years without regard to their effect on vector-borne diseases. Similarly, tropical forests are being cleared at an increasing rate, and agricultural practices such as rice production have also increased. Climate change (e.g. global warming and El Niño Southern Oscillation) is often cited as the cause for the emergence/resurgence of vector-borne diseases, especially malaria, dengue and yellow fever (Gubler 1998).

6 Seasonal Prevalence of JE Vectors in Correlation with Meteorological Parameters—A Case Study in Andhra Pradesh

JE is a well-known vector-borne disease in tropical and sub-tropical countries and is alarming to the human population. JE viral prevalence is mostly reported in the Southern states of India. From the past reports, it is assumed that the occurrence of JEV is directly associated with various factors like congenial environmental conditions, nature of agricultural practices, vector density and the reservoirs. Different control operations have been implemented through various agencies but checking the outbreaks of this virus has proven difficult. The overall disease spectrum is poorly understood. Hence, the main aim and objectives of the study being described is to collect detailed information regarding transmission dynamics of JE from the

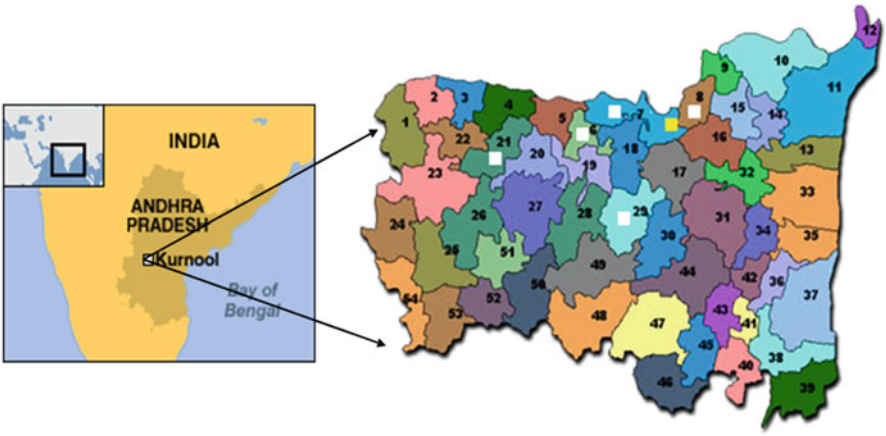


Fig. 6.4 Kurnool map

rural and urban areas of Kurnool district of Andhra Pradesh. To conflate the weightage of various factors authorizing the endemicity of the disease, a Bayesian network-based method was implemented with the following objectives for forecasting the JE vector density well in advance.

- To study the bionomics and seasonal prevalence of JE vector in rural and urban areas of Kurnool district of Andhra Pradesh.
- To study the influence of meteorological parameters on JE prevalence.
- To study the transmission dynamics of the JEV.
- To control transmission of JE by using specific bioinformatics tools.

Kurnool district has a population of 3,512,256 (urban: 792,654, rural: 2,719,602). Males constitute 51% of the population and females 49%. Kurnool has an average literacy rate of 63%, which is higher than the national average literacy rate of 59.5%: the males have a literacy rate of 69%, and the female literacy rate is 57%. In Kurnool, 13% of the population is under 6 years of age. Climatically, Kurnool district is generally warm and humid during most of the year, which can be divided into three periods. The dry period runs from February to June, intermittent rainfall occurs from July to October and winter months range from November to January. The Southwest monsoon gives more dependable rains in the area, which begin in June and persist to October. The monthly total rainfall for the last decade ranged from 3.5 to 241 cm. Most of the population is engaged in agricultural practices. Patchy paddy fields and waterloggings are commonly seen near the villages all over the district. Domestic animals like cattle, pigs and poultry commonly share their living with human dwellings. People in villages are economically poor and their houses largely consist of one or two rooms with a thatched roof.

Out of the 69 Public Health Centres (PHCs) of Kurnool district (Fig. 6.4), six areas (five villages and one urban area) with the highest number of JE cases since 1996 were selected: Peddathumbalam, Nandanapalli, Nandikotkur, Gudur, Cherukulapadu and

Kurnool (urban). A total number of seven fixed catching stations (seven households × six study areas) were identified using the random selection procedure for mosquito collection every month. The entomological, epidemiological, agriculture and meteorological surveys were conducted from these selected study areas between June 2001 and July 2006.

6.1 Entomological Studies (Vector Surveillance)

Vector surveillance was initiated in all the six index areas to know the seasonal prevalence of JE vectors. The mosquito population was sampled during dusk hours (6.30–7.30 PM). Indoor and outdoor resting mosquitoes were collected from cattle sheds (fixed catching places) every month with the help of mechanical aspirators (Hausherr's Machine Works, NJ, USA). Light traps were also used for sampling vector populations. The collected mosquitoes were separated species wise. The relative density of female *C. gelidus*, *C. tritaeniorhynchus*, *C. quinquefasciatus*, etc., was recorded as the number of females collected per man-hour (PMH). The collected mosquitoes of *C. gelidus*, *C. tritaeniorhynchus*, etc., were segregated into different pools containing 50 mosquitoes in each pool. These pools were transported to a laboratory (in liquid nitrogen cans) for analysis of JEV. The mosquito density (PMHD) was calculated by using the following formula.

6.2 Analysis of JEV from Mosquitoes

A total of 974 mosquito pools (30,075 mosquitoes) of *C. tritaeniorhynchus* and 594 mosquito pools (20,070 mosquitoes) of *C. gelidus* were preprocessed for virus infection by following two different complementary systems.

1. Antigen capture enzyme-linked immunosorbent assay (ELISA): the procedure followed was that of Gajanana et al. (1995), using monoclonal antibody 6B4A-10 (reactive against all the viruses in the JE–WN–SLE–MVE complex) as capture antibody and monoclonal antibody peroxidase conjugate SLE MAB 6B6C-1 (reactive against all flaviviruses) as detector antibody.
2. Insect bioassay: mosquito pools positive in ELISA were inoculated intracerebrally into *Toxorhynchites splendens* Wiedemann larvae, incubated at 29°C for 7 days on head squash preparations (Toxo-IFA). Smears were screened using a JEV-specific monoclonal antibody, MAB 112 (Mourya et al. 1989), and detected by antimouse immunoglobulin conjugated with fluorescein isothiocyanate (FITC) (Dakoppats, Denmark).

6.3 Minimum Infection Rate

The virus infection rate in mosquitoes was expressed as minimum infection rate (MIR) per 1,000 females tested. The MIR was calculated by using the following formula.

$$\text{MIR} = \frac{\text{Number of positive pools}}{\text{Number of mosquitoes tested}} \cdot 1{,}000$$

6.4 Serological Survey

To ascertain the status of JEV activity in the human population, sero-epidemiological studies were undertaken in the study areas of Kurnool district. The blood samples were collected from school children (6–10 years old) through finger prick method. The collected blood samples were centrifuged to separate the sera. The sera samples were transported to a laboratory in liquid nitrogen cans for further analysis. Sera of school children were examined by the haemagglutination inhibition (HI) test following the protocol of Clarke and Casals (1958), for identifying flavivirus antibodies (JE/West Nile/Dengue).

Along with the entomological and epidemiological details, other details like agriculture in hectares, paddy cultivation in hectares, crops per year, crop seasons, irrigation sources, water stagnation period in paddy, types of fertilizers, summer crops, piggeries, cattle sheds, positive JE cases/deaths and vaccination details have been collected from the selected study areas.

6.5 Meteorological Conditions in Study Areas

The climate of Kurnool is tropical, and it can be divided into three seasons: dry (February–June), wet or monsoon (July–October) and winter (November–January). The Southwest monsoon gives more dependable rains in the area, which begins in June and persists through October. During the study period (2002–2006), the maximum temperature ranged from 30.3°C (December 2005) to 42.8°C (May 2003). Two suburban localities and six villages where at least one JE case occurred during 1997–2002 were selected as index areas and sampled once every 2 months. Meteorological conditions such as temperature (maximum and minimum), relative humidity (8.30 and 17.30 h), rainfall and wind speed of the study areas were collected from the Indian Meteorological Department, Hyderabad.

6.6 Agricultural Details

Details of agricultural output of the study area in hectares, paddy cultivation in hectares, crops per year, crop seasons, irrigation sources, water stagnation period in paddy, types of fertilizers, summer crops, piggeries, cattle sheds, positive JE cases/deaths and vaccination details have been collected from the selected study areas.

6.7 Results

The study sites (rural/urban) with different ecological conditions showed striking differences in relative abundance of the JE vectors. *C. gelidus* was the most abundant species in urban areas, making up 68.1% of the total mosquitoes collected, followed by *C. tritaeniorhynchus* (25.7%), whereas in rural areas *C. tritaeniorhynchus* was the most abundant species, making up 57.5% of the total mosquitoes collected, and *C. gelidus* consisted only 2.5% of the total. *C. tritaeniorhynchus* population fluctuates in a similar pattern in both rural and urban areas (Figs. 6.3 and 6.4). Abundance of *C. tritaeniorhynchus* was lowest in summer, and it increased from September onward coinciding with monsoon and rice cultivation. *C. tritaeniorhynchus* abundance did not show significant correlation with meteorological factors except for temperature in rural and urban areas. Correlation analysis of abundance of *C. tritaeniorhynchus* with temperature showed a significant negative relationship in rural areas ($r = -0.50$, $P < 0.01$) and in urban areas ($r = -0.49$, $P < 0.01$). It showed that the *C. tritaeniorhynchus* population is strongly affected by the availability of suitable breeding habitats.

Epidemiologists concerned with the prevention of JE have long suggested positive correlations between the number of JE cases and weather conditions. Local health authorities in endemic areas warn the public in early summer of the possibility of a JE epidemic if the local meteorological agency forecasts a hot summer. This is because it has been commonly believed that hot summers result in large epidemics of JE due to heightened reproductive activities of the vector mosquito, *C. tritaeniorhynchus*, on the one hand, and higher infection and morbidity rates among people who have become more susceptible due to fatigue, on the other (Mogi 1984). The cogency of the prediction of epidemics based on meteorological factors necessitates a statistical outlook.

6.8 Impact of Weather Variables on JE Vector Density

It is crucial to study the impact of weather on the transmission of JE, as global warming might change the pattern of temperature and rainfall which may directly or indirectly influence mosquito density (Murty et al. 2010). Through this study, it was inferred that there is a significant difference in *C. tritaeniorhynchus* density (PMH) in

Table 6.1 Mean weather variables of Kurnool district during the study period (July 2002 to May 2006)

Weather variables	Mean ± S.D. ($n = 12$)
Rainfall (mm)	62.395**
Wind speed (km/h)	3.137 ± 1.691**
Maximum temperature (°C)	34.538 ± 3.610**
Minimum temperature (°C)	22.826 ± 3.532**
Maximum RH (%)	69.664 ± 8.384*
Minimum RH (%)	42.774 ± 11.761*

S.D. Standard deviation
$^{*}p < 0.01$; $^{**}p < 0.001$

the rural and urban areas during different seasons of a year. The *C. tritaeniorhynchus* population was more in the rural area than in the urban areas, in all the seasons except from July 2002 to September 2002. So, it is assumed that irrespective of the seasons *C. tritaeniorhynchus* is more abundantly found in the rural areas. *C. gelidus* were found mainly confined to the urban areas and very low numbers of *C. gelidus* were captured in rural areas except during November–January. Hence, it was observed that seasonality appeared to be the major influencing factor in comparing the densities of the two mosquito species. In the seasonal abundances, the PMH density ranged from 100 to 250 for *C. tritaeniorhynchus* and *C. gelidus* during the study period. The mean values of all the meteorological parameters for different seasons of the year are mentioned in Table 6.1. Optimum temperature was between 22.8°C and 34.53°C ($p < 0.001$); relative humidity (RH) between 42.77 and 69.66% ($p < 0.01$) and rainfall reaching 62.395 mm ($p < 0.001$) were observed throughout the study period. These factors are generally conducive for mosquito breeding and proliferation.

It has been observed that temperature at 28°C with 50–55% RH is more favourable for elevation in mosquito density than the condition of lower temperature with higher humidity (22°C/80–85% RH). In the present investigation also, the temperature was between 22°C and 34°C with lower to medium humidity (42.7–69.6%), which might have facilitated the higher mosquito density in both rural and urban areas.

6.9 Summary of Seasonal Patterns of JE Vectors

In Kurnool, the JE epidemic season begins in August and peaks in November, and then it declines dramatically in December. Our entomological investigations have improved our understanding of the seasonal patterns of JEV transmission, which is highly seasonal in Andhra Pradesh. The results indicated that *C. tritaeniorhynchus* abundance was highest during JE epidemic months, and JEV infections in vector mosquitoes were found only during these months. MIR and maximum likelihood estimates of JEV infections of *C. gelidus* were lower compared with the values obtained for *C. tritaeniorhynchus*, which indicate the primary role played by *C. tritaeniorhynchus*.

7 Japanese Encephalitis-Bayesian Network: A Forecasting Model for JE Vectors—A Case Study in Andhra Pradesh

The major constraint in public health is to understand the transmission dynamics of a disease, and to control vector proliferation and disease morbidity. Even though several control strategies are in practice, diseases are still re-emerging and threatening the human population (Gubler 1998). There are also other factors that facilitate the fast growth of mosquito population in any locality such as unplanned urbanization, poor sanitary conditions, improper planning for implementation of control programs and lack of realistic knowledge of disease transmission (Ramaiah et al. 2000; Snehalatha et al. 2003). It is also observed that vectors acquire resistance towards insecticides. Many integrated approaches for control of mosquitoes and mosquito-borne diseases were implemented at the field level, but most of them were not durable and re-emergence of mosquitoes was found in all the endemic places. One of the most important factors for effective control or total eradication of mosquitoes is effective communication between the root level worker and administrators. To avoid the above pitfalls there is an urgent need for a new approach for implementation of control strategies, with partnerships between available skilled personnel and administrators to effect swift decisions regarding effective control of vectors and vector-borne diseases.

7.1 History and Progress of JE Models

Protecting people from vectors and vector-borne diseases demands special care and a significant increase in resources. Vector-borne diseases are among those infectious diseases causing the highest disease burden today, and may be expected to represent the highest proportionate disease burden in the future. The incidence of vector-borne diseases is grossly disproportionate, with overwhelming impact in developing countries located in tropical and sub-tropical areas. JE, a major vector-borne infection, kills many people throughout the world, particularly in developing countries like India. Modelling is a powerful tool that can be used to control JE and other infectious diseases effectively. Hence, the present study is focusing on disease models that were developed using computational (data-mining) techniques like artificial neural networks (ANNs), principal component analysis (PCA), support vector machines, etc., statistical methods, mathematical, dynamical, spatial modelling and GIS/RS mapping by using various parameters.

Dynamical systems are employed in numerous circumstances to model infectious diseases. These models lead to a better understanding of the causes, distribution and control of diseases. Public health officials use these models in decision-making. A number of interesting consequences can be understood by constructing simple models using ordinary differential equations. One of the first susceptible–infective–recovered–susceptible (SIRS) models in ordinary differential equations with constant recruitment and disease-induced death for the spread of an infectious disease in the population was developed by Anderson and May (1979).

The human is a dead-end host for JE infection; since the disease is transmitted by a vector (mosquito) population, there are tremendous seasonal fluctuations of the disease and vector populations in different seasons. To capture these seasonal fluctuations, a regression-equation model using a third-order harmonic Fourier series with a linear trend has been used by Mukhopadhyay et al. (1993) to simulate the pattern of monthly occurrences of JE. Considering that some portion of the immune reservoir population is also infective, two differential-equation models involving human and reservoir populations only (Mukhopadhyay and Tapaswi 1994; Tapaswi and Mukhopadhyay 1995) have been proposed assuming constant birth rates in the former and density-dependent birth rates in the latter, and constant population sizes in both the populations. A three-population model involving vector, reservoir and human populations with logistic growth of the vector was considered (Tapaswi et al. 1995), and endemicity of the disease was investigated at the global level. A lot of experimental work on JEV in Japan had been performed by Scherer et al. (1959). A two-population model of JE spread involving variable reservoir and human populations assuming a constant equilibrium size of the vector population has been considered by Ghosh and Tapawsi (1999), and they had investigated the dynamics of this dreaded disease in a two-population system, consisting of reservoir and human populations only.

The transmission model considered for the spread of JE in a human population of varying size from a reservoir population (pigs, cattle, equines, birds, etc.) through a vector population (particular species of mosquitoes) is of SIRS type for the human and reservoir populations and susceptible–infective–susceptible (SIS) type for the vector population. The study considered the logistic differential equation with density-dependent birth rate for the vector population, whereas the reservoir population is of constant size. They assume that the human population is regulated by the disease and also assume that there is a constant recruitment rate of susceptible individuals into the human population (Tapaswi et al. 1995).

Spatial modelling and spatial statistics are tools to analyze and integrate the spatial component in epidemiology of vector-borne disease. Research, surveillance and control programs based on landscape ecology consider the spatial heterogeneity of biotic and abiotic components as the underlying mechanism, which determines the structure of ecosystems. The aim of this application is to identify the exact endemic location based on the actual information and also develop disease forecasting and geo-spatial data modelling in order to take necessary steps to reduce the cases.

Recent papers have used ecological niche modelling programs, e.g. Maxent and GARP, to predict the distribution of disease vectors (Peterson and Shaw 2003; Moffett et al. 2007). In this study, researchers used the Maxent program to model the distribution of *C. tritaeniorhynchus* in the Republic of Korea. Using mosquito collection data, temperature, precipitation, elevation, land cover and SPOT normalized difference vegetation index (NDVI) models were created. Output maps from the models matched several known ecological characteristics of this species' distribution. The model demonstrated low probabilities for forest covered mountains, which corresponds to findings in the literature that *C. tritaeniorhynchus* is infrequently found above 1,000 m altitude.

The statistical models are applied to yield a predictive program for the occurrence of JE on the island of Taiwan. Four different forms of the model are evaluated: linear

regression, log-linear regression, logit models and discriminant analysis. Of the models employed, it was found that linear regression produces the best results. A significant correlation was found between observed and predicted disease incidence rates (correlation coefficient = 0.75). No systematic residual biases were observed in the final predicted results.

Information technology (IT) and computational modelling plays a vital role in the improvement of the environmental hygiene (Novak and Hamel 1999). Many workers have tried using IT for control of vector and vector-borne diseases (World Health Organization 1965). Entomological and epidemiological databases, decision rules (Liao 2003) and prediction models (Raddatz 1986; Yang et al. 2002) have all been used for control of malaria (Murty et al. 2006), microfilaria (Carabin et al. 2003), malaria using Bayesian network (Cancre et al. 2000) and prediction of defervescence fever of dengue and dengue haemorrhagic fever patients. Using ANNs (Ibrahim et al. 2005), and impact of environmental factors on *Anopheline* mosquitoes using data-mining methods (Sweeney et al. 2007), geographical information system (GIS) applications have been used for the control of malaria (Sipe and Dale 2003), filariasis (Hassan et al. 1998) and dengue (Tran et al. 2004). Furthermore, hierarchal categorization of endemic areas and dissemination through the WWW (Novak and Hamel 1999; Stephen and Berger 2001) were being widely used for many infectious diseases.

Significant changes in IT are taking place in many sectors. Health professionals need to maximize the potential benefit of the evolving information technologies as a means for improving public access to information and care (Wallace 1997). With continuing advances in IT, the application of computers in medicine has increased rapidly, and they have the potential to revolutionize health care systems.

Among these, database management systems and artificial intelligence (AI) tools are being used extensively in vector biology (Szolovits 1982). AI contributes in several areas such as knowledge acquisition, knowledge representation, reasoning, critiquing, explanation capabilities and insight into human cognitive processes in problem solving (Barr and Feigenbaum 1982; Shortliffe 1993; Uckun 1993; Lillehaug and Lajoie 1998), while a database management system is a fundamental step for developing the different models such as forecasting models, prioritization of endemic zones and disease surveillance systems (Miyaki et al. 2002). Hence, in this study an attempt has been made at the application of Information Technology in the development of a database management system of JE and a forecasting model for the prediction of the density of JE vector using a Bayesian network.

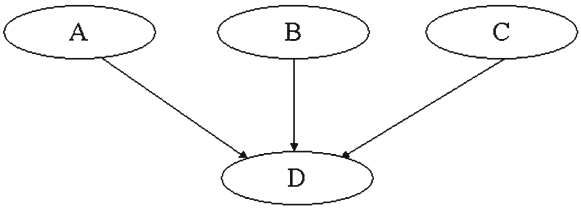
7.2 Aims and Objectives

In this study, an attempt has been made to develop different Information Technology applications for entomological and epidemiological aspects and to develop a forecasting system for JE. Components of this are as follows:

- Development of a user-friendly relational database management system (RDBMS) for JE
- Bayesian network applications for the prediction of JE vector density (JEBNET)

7.3 Prediction Engine

The Japanese Encephalitis-Bayesian Network (JEBNET) algorithm used for this study is an extension of Bayesian networks. It attempts to make a prediction in three phases.



Phase-1: This is the classical Bayesian network phase. To predict a child node in the Bayesian network, the program checks the values of the parent nodes provided and examines the database looking for all instances where the pattern of the values taken by the parent nodes matches the given set.

Phase-2: This is an extension phase and is used because the limited size of the database makes it highly probable that a given assignment tuple of values of the parent nodes has not been perceived before. In such a case, compute the value of the child and measure how much is necessary to disturb a state for which we know the exact solution. Next, how much this perturbation of the values of the parents affects the child node in general over the entire database was taken into account. Suppose the value of the child changes by the tuple A, and with the known pattern the value of the child is represented by the tuple B. Then the projected value of the child is given by the tuple A+B.

Phase-3: This final phase begins when the database contains neither a single record with the required assignment tuple for the parent nodes nor a single pair of records whose assignment tuples for the parent nodes vary by the required difference tuple. In this case, the difference tuple is split up into its components in the various coordinates, and each difference is treated as a separate difference tuple. The shifts computed for the separate difference tuples are all added up to obtain the resultant shift, which is then added to the base value to obtain the predicted value.

7.4 Application Outputs

For the analysis using a Bayesian network, month, wind speed, rainfall, relative humidity, maximum temperature, minimum temperature, irrigation, agricultural area, area for paddy cultivation, water depth in paddy fields, piggeries and cattle sheds records were considered in predicting the density of mosquito population. The network primarily contains the information about relationship and interdependence variables. The Bayesian network designed for the above-collected parameters is shown in Fig. 6.5.

The algorithm used in this model to predict the PMH of JE mosquitoes is a slight modification of the Bayesian network algorithm and the preprocessing system is implemented on a PC platform using JAVA programming language. Figure 6.6

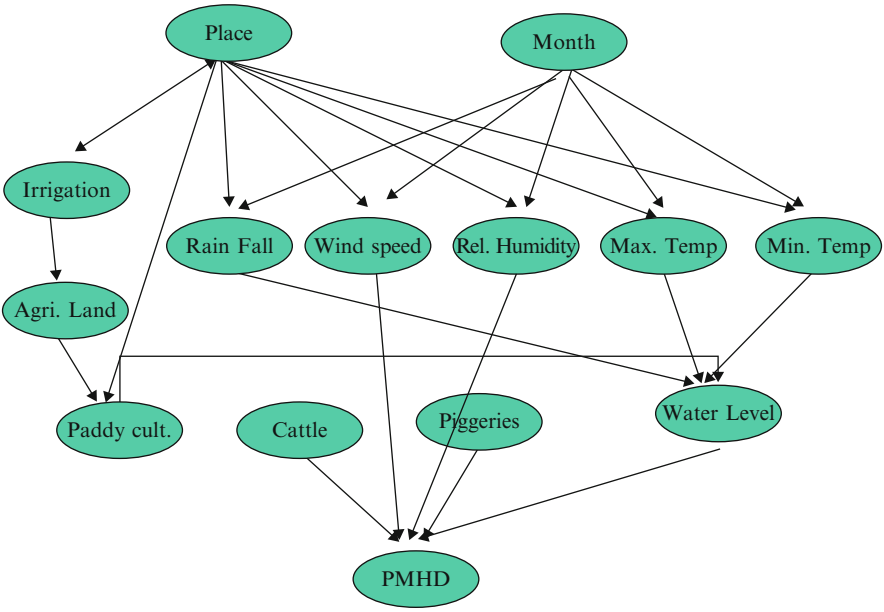


Fig. 6.5 The structure of the Bayesian network—JEBNET model

shows the main window where the user may select the Bayesian belief network file, data files, selection of agriculture, meteorological parameters and test data files.

This algorithm attempts to calculate the mosquito population that can occur with maximum likelihood. This likelihood of an event is calculated as follows:

$$\text{Likelihood of an event } A = \frac{\text{Number of cases in which the event } A \text{ occurs} \dfrac{n!}{r!(n \square r)!}}{\text{Total number of cases in the database}}$$

The JEBNET model uses a data file that contains the different parameters specified in the belief network file. The network file contains meteorological, agricultural and animal parameters. The model was tested in a variety of ways to verify its value in all conditions. The test involved testing the overall performance of the model in terms of prediction of PMH and its accuracy. JEBNET also provided the percentage of accuracy of the prediction by species wise and the results Table 6.2.

This tool helps in predicting the number of positive cases in the district, which can be reduced to negligible levels by taking preventive measures during the peak time of vector density. It takes advantage of a variety of data sources including agricultural, meteorological and animal data that were used to provide the density of vectors well in advance, which help in effective control of disease outbreaks. Considerable control measures can thus be implemented during the extrinsic incubation in mosquito as well as the intrinsic period when the humans are being infested by the mosquitoes. The advanced software tool can therefore forecast the vector abundance well in advance to alarm the vector control measures consequently.

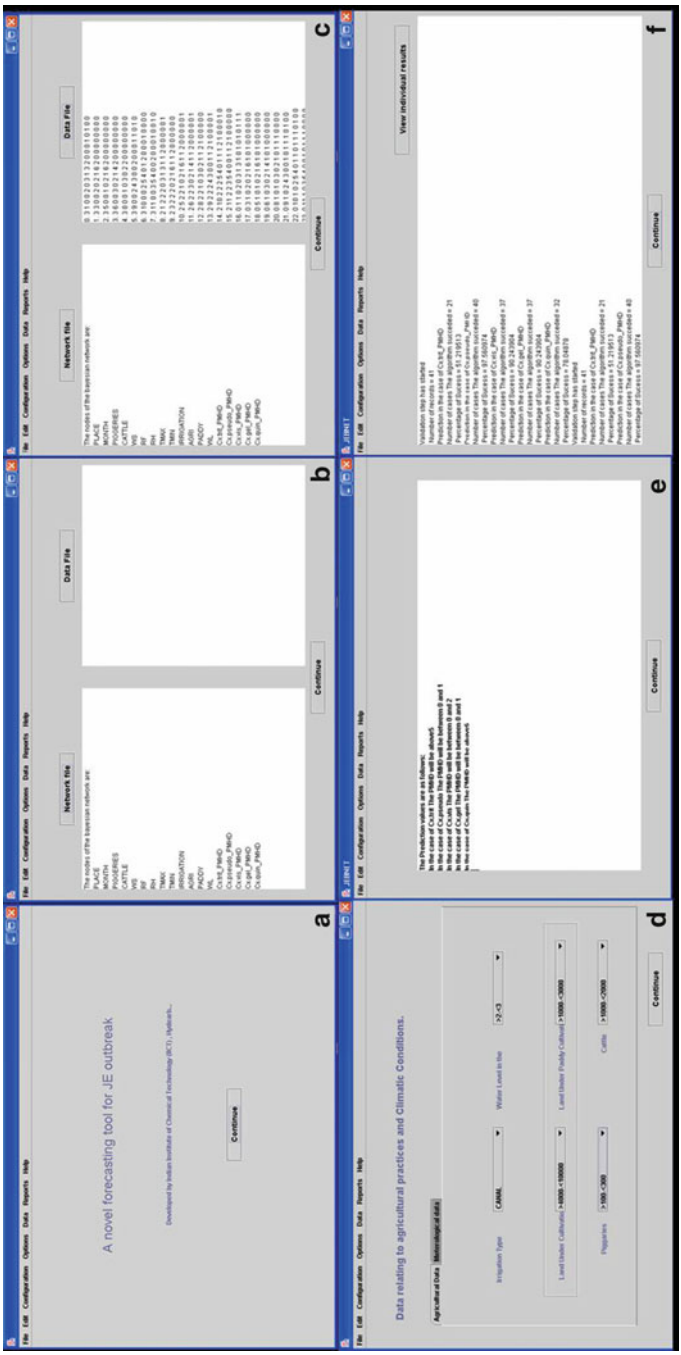


Fig. 6.6 The main windows of JEBNET. (**a**) JEBNET home page; (**b**) selection of Bayesian belief network file; (**c**) selection of data file; (**d**) agricultural and meteorological details selection; (**e**) prediction results of JEBNET; and (**f**) comparison of results with test data

Table 6.2 Percentage of accuracy of prediction species wise with results

Mosquito type	Test cases	Correct cases	Percentage of accuracy
Culex tritaniorhynchus	41	30	73.17
Culex psuedovishnui	41	39	95.12
Culex vishnui	41	37	90.24
Culex gelidus	41	36	87.80
Culex quinquefasciatus	41	30	73.17

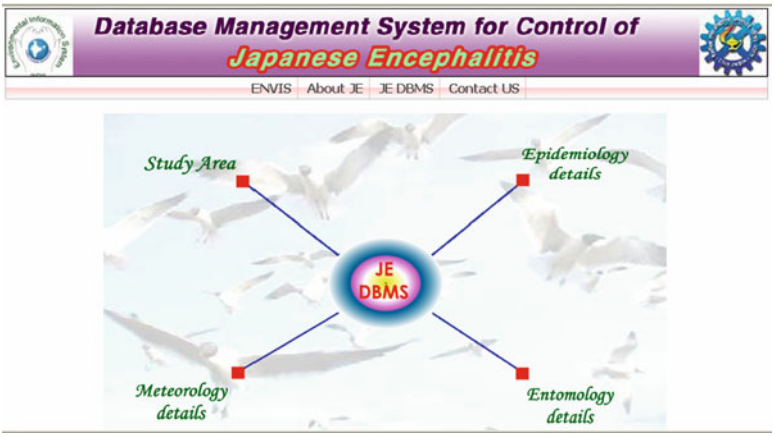


Fig. 6.7 Japanese encephalitis-database home page

8 Japanese Encephalitis-Database Management System

A thorough survey of entomologic, epidemiologic and meteorological factors pertaining to a disease is necessary to implement control measures accordingly. This necessity for epidemiological surveillance has taken advantage of modern technologies in information management and exchange and dissemination through the Internet and World Wide Web connectivity (XII Information dissemination; NIHS). Thus by utilizing the web-based tools and encompassing the enormity of data through databases like Microsoft Access, a "Database Management System for JE" has been developed and hosted through the URL: http://iictenvis.nic.in/DBWeb/index.htm (Fig. 6.7).

8.1 Design

This dynamic system unraveled the difficulties in the conventional survey and data management method by the maintenance of unique forms, one each for "Study Area details","Entomology Details","Epidemiological Details (Human)","Epidemiological

Details (Animal)". Public health professionals, medical researchers and even a layperson from any region could enter the details in these forms based on the data that they have accrued. On submission of these forms, these details are integrated to MS-Access tables. The front-end program for this DBMS includes "Active Server Pages (ASP)" to convey the data submitted through the forms to MS-Access, the back-end product. It is an effective way of assisting the existing control operations. The split database architecture of Ms-Access assures a secure functioning at the back end and therefore depending on the requirement of incorporating new details or changing the interfaces, the front-end forms could be redesigned with ASP.NET.

8.2 Output and Utility

In the technical context, this serves the purpose of straightforward analysis of entomological and epidemiological indices in real time as the DBMS is web based and acts as a dynamic system to direct and regulate the control programs without interruption. For any vector control, the epidemiological, entomological, meteorological and socio-economical data from various resources forms the baseline for the program. This cosmic data is being incorporated into this database management system with ease in storing, managing and manipulation. The database is a large organized body of persistent data, associated with computerized software designed to update, query and retrieve components of the data within the system. MS-Access packages are ideally suited for information retrieval and time-to-time updating of the data. In such a way, it ascertains an effective way of assisting the existing control operations for the disease. This Japanese encephalitis-database management system (JE-DBMS) has unlimited scope across all geographies without perturbing its framed objectives. Therefore this web-based database could be utilized thoroughly for making decisions on vector control.

9 Ratiocinations

Any disease control program is a cumulative effort of researchers, health care personnel and the communities. Awareness of epidemics helps in planning control strategies or preventive measures well in advance, which requires effective and efficient information dissemination and exchange. JE, with no specific antiviral therapy, requires an advanced apprehension of control and management procedures. Hence a precise panorama of the aetiologic and entomologic orbits of this vector-borne viral infection is essential for medical and public health professionals. In this way, the computational methods and sure-shot correlations and predictions deriving from this study could be ranked high in the innovations of JE vector management.

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Chapter 7
Filaria Monitoring Visualization System: A New Dimension for Integrated Control of Lymphatic Filariasis

U. Suryanarayana Murty and Jianhong Wu

The nineteenth and twentieth centuries had been exciting times for investigation of transmissible diseases. Pathogens were cultivated from diseased humans and animals, vaccines were developed and immunity studies carried out for use in diagnosis and prevention of disease. Koch's postulates were promulgated and used to prove that various microorganisms were the cause of diseases. Quarantine was practiced to prevent the spread of diseases that were transmitted directly from one person to another. Antibiotics and other chemotherapeutic agents have been improved to suppress many serious diseases. Despite these successes, failures lurked in the background. The dark shadow was cast on many vector-borne diseases, which had complex epidemiology and reservoirs in various animals other than humans, and flared up unpredictably. The vector-borne agents include the whole spectrum of infectious agents: viruses, rickettsia, bacteria, protists and helminths. Except for the blood flukes (*Schistosoma* spp.), most of the disease agents were found to be transmitted by arthropods viz., lice, bugs, mosquitoes, black flies, midges, sand flies, ticks and mites. Among all the blood-feeding group of insects, mosquitoes are by far the most important from the standpoint of human health, responsible for transmission of many pathogens that cause mortality and morbidity.

Towards the end of the nineteenth century, scientists discovered that a number of diseases were transmitted by arthropods. As early as 1848, Joseph Nott first proposed that yellow fever and malaria were transmitted by mosquitoes. In 1878,

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Manson showed that the mosquito *Culex quinquefasciatus* (Diptera: Culicidae) transmitted roundworm to humans. In 1897, Sir Ronald Ross showed that malaria was transmitted by mosquitoes. Mosquitoes are a well-known group of insects that annoy man and transmit several human diseases. They are the only vectors of pathogens causing filariasis, malaria, Japanese encephalitis, dengue, yellow fever, Chikungunya fever and several other diseases in humans. Insect pathogens typically must infect and replicate or develop in both vector and a vertebrate host. Today a number of arboviruses, four species of human malarial parasites and two species of filarial parasites remain as leading causes of human mortality and morbidity in the world.

Health is a cherished human value shared across geopolitical and sociocultural divides. It is thus a sad indictment on our global morality that despite enormous biomedical advances and global economic prosperity in the past few decades, huge disparities in health status persist between and often within countries. Thirty per cent of the global burden of disease and a quarter of all deaths are still attributed to infectious diseases, and more than 95% of these deaths occur in the developing world where poverty is prevalent (Gwatkin et al. 1999; Folch et al. 2003). Lymphatic filariasis (LF) is a neglected tropical disease and particularly interesting case in point. This parasitic infection, one of the six diseases that had been considered potentially eliminable, was endorsed for global elimination by the World Health Organization (WHO) in 1997. A global elimination programme, using annual mass drug administration (MDA), was established in 2000 (Ottesen et al. 1997). Despite encouraging early expansion of the global LF elimination programme, it has become apparent that the donation of drugs alone will not be sufficient to ensure that all individuals at risk receive necessary treatment to interrupt transmission. Most notably, no additional African countries have embarked on mass campaigns in the past 2 years due to lack of funds for operational delivery of programme activities.

Lymphatic filariasis is thought to have affected humans since approximately 1,500–4,000 years ago. The first documentation of symptoms occurred in the sixteenth century, when Jan Huygen Linschoten wrote about the disease, during the exploration of Goa. After the exploration of other parts of Asia and Africa, he turned up with further reports of disease symptoms. It took some centuries after those initial observations for the better understanding of the disease to develop. In 1876, Joseph Bancroft discovered the adult form of the worm, and finally in 1877 the life cycle involving an arthropod vector was theorized by Patrick Manson, who proceeded to demonstrate the presence of the worms in mosquitoes. In 1900, George Carmichael Low determined the transmission of the worm in the proboscis of the mosquito vector. Lichentenstein and Brug first recognized *Brugia malayi* as a distinct pathogen in 1927. They reported the occurrence of a species of human filaria in North Sumatra that was both physiologically and morphologically distinct from the *Wuchereria bancrofti* microfilaria commonly found in Jakarta and named the pathogen *Filaria malayi*.

1 Historical Review of Lymphatic Filariasis

Lymphatic filariasis is commonly known as elephantiasis and is considered a major public health problem due to its considerable morbidity and social stigma. It is a painful and profoundly disfiguring disease that has major social and economic impact in many parts of the world (Ottesen et al. 1997). The disease-affected regions are mainly in Asia, Africa, the Pacific Islands, several of the Caribbean Islands and South America (ITDE 2009). Lymphatic filariasis was first described by the Egyptians in 2000 BC. An ancient statue of Pharaoh Mentuhotep II shows swollen limbs, a common sign of elephantiasis. Other early evidence of filariasis includes artefacts from the Nok civilization in West Africa dating back to 500 AD, depicting scrotal swelling, also a common finding in lymphatic filariasis (Grove 1990). Filariasis is known to be caused by three types of nematode worms, *W. bancrofti*, *B. malayi* and *Brugia timori* belonging to the order "Filariidae" (Sasa 1976). More than 100 species of mosquitoes are known to involve in the transmission of lymphatic filariasis out of which, *C. quinquefasciatus* (Say 1823), commonly called the Southern house mosquito, is the most formidable and widely prevalent vector for Bancroftian filariasis.

600 BC	Susruta	Described elephentoid leg as "stone legs" or "shlipadam"
700 BC	Madhavakara	Described clinical manifestation of filariasis
1709	Clark	Gave the name "Malbaer Leg"
1863	Demarquay	Microfilaria (MF) in hydrocoele fluid
1866	Otto Wucherer	Microfilaria (MF) in chylus urine
1870	Richard Lewis	Microfilaria (MF) in peripheral blood in Calcutta
1876	Bancroft	Rediscovered adult female worm in Brisbane, Australia
1877	Cobbald	Proposed the scientific name *Filaria bancrofti*
1877	Manson	In China, discovered the development of *W. bancrofti* parasite in *Culex* mosquitoes
1878	Manson	Described the periodicity of MF associated with feeding habits of mosquitoes
1910	Manson-Bahr	Absence of periodicity of *W. bancrofti* in Polynesia
1927	Brug	Discovered MF of *B. malayi* in Indonesia
1931	Brug & Delook	Discovered "*Mansonia*" mosquitoes as efficient vectors of *B. malayi*
1940	Raos and Maple Stone	Discovered adult worm of *B. malayi* in India
1947	Hewitt et al.	Discovered diethylcarbamazine (DEC) as an effective filaricide
1950	Hawking et al.	Demonstrated the mode of action of DEC on the parasite
1955–1956	NFCP	National Filaria Control Programme (NFCP) was launched in India

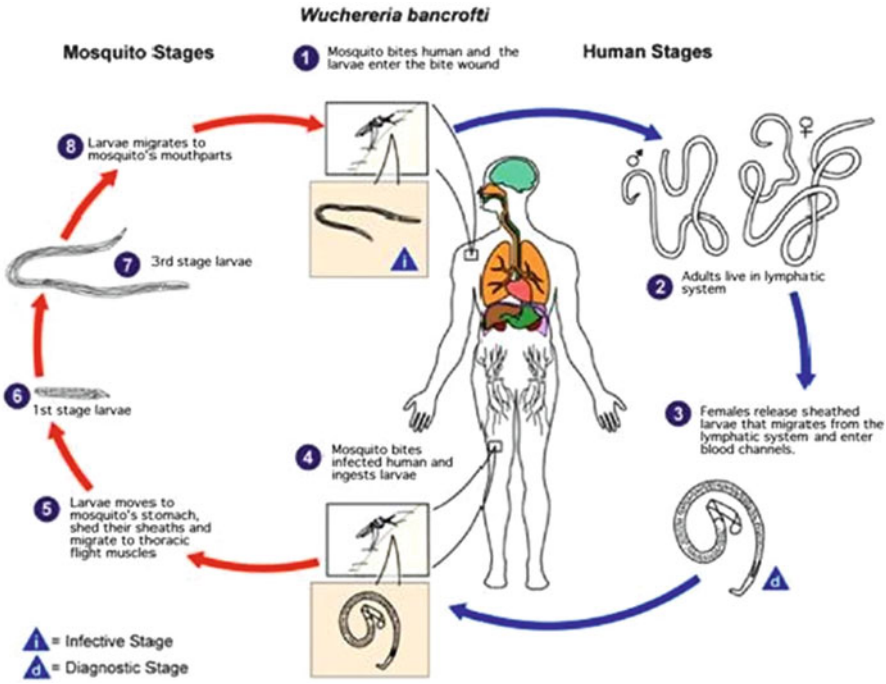


Fig. 7.1 Life cycle of *Wuchereria bancrofti*. *Source*: CDC

1.1 Life Cycle of Lymphatic Filariasis

The life cycle of the parasite involves a definitive host, an intermediate host and the mosquito (Fig. 7.1). The adult filarial worms live in the lymphatic system of man. The male worms of *W. bancrofti*, after mating with female adult worms produce a large number of embryos called "microfilariae (MF)". In the female mosquito body, the MF penetrate the gut wall and migrate to the thoracic region, where they develop into L1, L2 and finally into L3 or infective stage within 10–12 days under tropical climatic conditions (Krasfur and Garrett Jones 1977; Rajagopalan et al. 1977). Most of the infective stage larvae are found in the head region of the mosquito (Paily et al. 1995). They escape through the proboscis of the mosquito and are transmitted to man during the subsequent blood meals of the vector mosquitoes. Within the human body the larvae migrate to the lymphatic system and develop into adult worms in about 8 months to 2.5 years (Hairston and Jachowski 1968; WHO 1992). The average life span of the adult worms is about 5–10 years (Vanamali et al. 1989, 1990) with a maximum longevity of 40 years (Carme and Laigret 1979). The average number of reproducing female worms per microfilaraemic person is estimated to be about 6.5 (Hairston and Jachowski 1968). An adult worm can produce millions of MF during its lifetime.

1.2 Vector of Lymphatic Filariasis

The principal vector of filariasis, *C. quinquefasciatus,* was first described by Say in 1823, whereas Wiedemann described the same species in 1828 and named it as *Culex fatigans*; it is commonly referred to as the Southern house mosquito. Even though the argument persists, Stone (1957) and Stone et al. (1959) considered *C. quinquefasciatus* to be the valid name. Sirivanakarn and White (1978) have designated a neotype male for the Southern Tropical house mosquito to promote nomenclatural stability concerning the interpretation and use of the name *C. quinquefasciatus* Say. Belkin (1977), Sirivanakarn and White (1978) stated that under the law of priority, the name *C. quinquefasciatus* Say (1823) takes precedence over all accepted junior synonyms, notably *C. fatigans* (Wiedemann 1828).

Species of *Culex*, *Anopheles*, *Aedes* and *Mansonia* are also reported as vectors for filariasis. More than 50% of the infections all over the world are transmitted by *C. quinquefasciatus* species (Southgate 1984) (Fig. 7.2). In many countries, including India, *C. quinquefasciatus* is highly anthropophilic and rests and feeds indoors as well as outdoors. Larvae and adults of *C. quinquefasciatus* are found at an altitude of about 2,130 m in Sri Lanka and also at an altitude of 500–1,850 m in the states of Himachal Pradesh and Jammu & Kashmir, India (Rao et al. 1973).

1.3 Epidemiology of Lymphatic Filariasis

Lymphatic filariasis is an important neglected tropical parasitic disease caused by nematode parasites of the genera Wuchereria and Brugia, in which the majority of global infections (some 90%) are caused by *W. bancrofti*. Both genera of parasites are transmitted by mosquitoes. The WHO has placed a number of people at risk in 83 countries at 1.307 billion (WHO 2006). The chronic and debilitating burden of LF maintains the cycle of poverty not only in infected individuals but also in entire

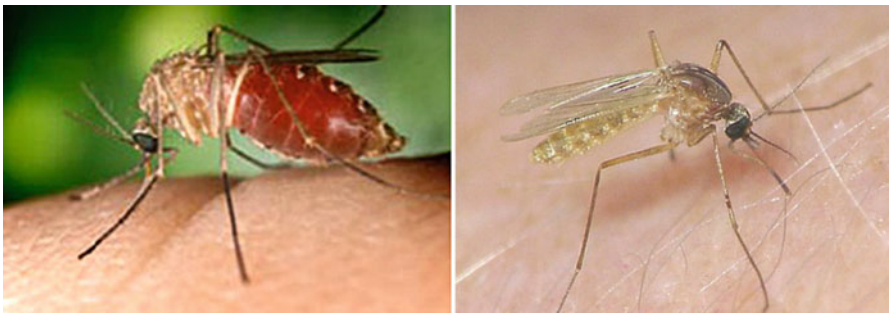


Fig. 7.2 *Culex quinquefasciatus* vectors for filariasis. *Source*: www.agriorganics.com and www.arbovirus.health.nsw.gov.au

endemic communities (Ramaiah et al. 2000). Indeed, as a disease of poverty, LF is endemic in 43 of the 50 countries classified as least developed nations (Galvez Tan 2003). India alone accounts for 40% of the global burden (Michael et al. 1996). The sub-Saharan Africa, Southeast Asia and the South Pacific islands and regions of the Americas are the other major endemic areas. Among the three parasite species, *W. bancrofti* accounts for 90% of the total disease burden and is very widespread. *B. malayi* accounts for 10% of the burden and is prevalent only in a few Asian countries. *B. timori* is found only in Timor and adjacent islands with limited prevalence.

1.4 Clinical Manifestations

Larvae migrate to lymph nodes and mature into adult worms over about 6 months. During this period, no microfilariae are found in blood, though subclinical disease and lymphatic damage may occur. Adult worms are relatively harmless; major symptoms result from the host's immune response to infection, which varies between individuals. Most patients present with acute attacks of "filarial fever" up to 15 months after infection. Lymphatic filariasis manifests as lymphedema of the extremities, genitalia, and breasts. The affected areas are swollen, painful, and often have a bad smell, with the skin turning warty and thickened with folds and cracks. Ulcers and swelling can grow large enough to interfere with movement and drastically debilitate victims (Burri et al. 1996; Shenoy 2008). This leads to disfiguration and is often associated with a very poor quality of life and chronic disability (Kalungi 2006).

Acute manifestations of lymphatic filariasis include acute adenolymphangitis (ADL) and acute filarial lymphangitis (AFL). ADL is the most common acute manifestation and is characterized by episodes of fever, inflamed lymph nodes in the groin and axilla, and localized areas of warmth, swelling, redness, and pain. These acute episodes are due to secondary bacterial infection and recur several times a year (Addiss and Brady 2007). The frequency of these attacks increases with the degree of lymphedema (Palumbo 2008). These attacks are also responsible for elephantiasis of the limbs and the external genitalia (Shenoy et al. 1999). AFL is rare and is observed when adult worms are destroyed in the lymph vessels or lymph nodes either spontaneously or by drug administration (Palumbo 2008). The most debilitating and disfiguring chronic manifestation of lymphatic filariasis is elephantiasis. This includes the severe swelling of the extremities, scrotum, vulva, and breasts, and occurs during the late stages of lymphatic filariasis. Elephantiasis occurs when the lymph vessels are blocked by nests of adult worms (Stanford University 2009).

1.5 Diagnosis

Microfilarial detection is a highly reliable method that was considered the diagnostic standard for many years and is still used in many regions. Microfilaria can generally be detected in the peripheral blood during the early stages of lymphatic filariasis,

even before clinical manifestations develop. However, once lymphedema is present, microfilaria are generally absent from the blood. Venous blood draw is usually done at night when microfilaria levels are highest and filtered through membrane filters, allowing for the identification of the microfilaria and quantification of the load of infection (Palumbo 2008). A dose of diethylcarbamazine (DEC) can also provoke the microfilariae to appear during the daytime if necessary (Molyneux 2009). After the collection of blood, nuclepore filtration is widely used as a concentration technique (Dickerson et al. 1990). Besides venous blood draws, capillary blood examinations can be conducted through finger pricks (Tolan et al. 2009). Capillary blood contains more microfilaria from *W. bancrofti* and *Brugia* than venous blood since parasites are more concentrated in the periphery (CDC 2009). A stained preparation can then differentiate the microfilarial species based on their morphologies. Delafield's hematoxylin stain is used to identify L. Loa, although the Giemsa stain is also often used to stain sheaths of other species (Cheesbrough 1998).

1.6 Control of Lymphatic Filariasis

Control of LF involves both preventing the spread of infection (transmission control) and alleviating the suffering caused by the disease (morbidity control). Transmission control can be achieved in two ways by reducing the vector population (vector control) and by reducing the intensity of blood microfilaraemia (parasite control) through drug administration.

1.6.1 Vector Control

Several measures are possible to control the vectors of filariasis. These include measures against adult mosquitoes and immature stages at the community level and personal protection at individual and household levels. Short-term vector control may not have much impact on the disease spectrum. Long-term vector control through residual spray of insecticides and especially malaria vector control tool is not preferred because of logistic, cost, and insecticide resistance problems. Larval control is a widely practiced method for vector control operations. Almost all types of antilarval measures viz., environmental, chemical, and biological methods play important roles because the vector breeding habitats are of wide variety and no single method may be suitable for all situations.

Larval control is the main stay of the National Filaria Control Programme (NFCP) in India. An integrated vector management strategy in Pondicherry, India, that envisaged environmental, chemical and biological methods reduced the *C. quinquefasciatus* density by 80–90% over a period of 5 years (Rajagopalan and Das 1987; Ramaiah et al. 1992). Polysterene beads were successfully used in the breeding habitats in India (Reuben et al. 2001). Vector control had also been successful in eliminating *W. bancrofti* from Solomon Islands and parts of Papua New Guinea,

where Anopheles species were the vectors of malaria and filariasis (Webber 1979, 1997; Bockarie 1994) and from Australia (Boreham and Marks 1986). While most of the vector control programmes still require both evaluation of their long-term impact and assessment of their cost-effectiveness (Ottesen et al. 1997), extension of larval control to the entire endemic areas in a large country like India may not be possible due to poor infrastructure and resource constraints.

Personal protection measures are gaining momentum in developing countries. Insecticide treated bed nets (ITBNs), coils and repellents are widely used, particularly in urban areas, where *C. quinquefasciatus* density is very high (Mulla 1968; Snehalatha et al. 2003). A recent study showed that use of ITBNs reduced the prevalence of microfilaraemia significantly (Croft et al. 2001; Bockarie et al. 2002). The impact of ITBNs in reducing malaria morbidity and mortality is encouraging; however, their value against lymphatic filariasis infection and disease is yet to be established.

1.6.2 Parasite Control

Parasite control aims at reducing the number of MF and adult worms in the human population and consequently the uptake of MF and transmission of infection by mosquitoes. The parasite populations can be controlled through selective or mass treatment. Selective treatment is expensive and cumbersome as it involves detection of all MF carriers and night blood screening of entire populations using invasive blood sampling procedures. In MDA all persons in a community, irrespective of their MF-status, are given treatment. Two anti-filarial drugs are generally used widely in mass treatment programmes viz., DEC and Ivermectin (IVR). However, co-administration of either of these drugs with Albandazole is recommended for MDA.

Both DEC and IVR are found to be very effective microfilaricidal drugs. Several clinical trials showed that a single dose of DEC (6 mg/kg body weight) or IVR (200–400 μ/kg body weight) can reduce MF intensity by 80–90% and these reduced levels can be sustained for about 1 year (Cao et al. 1997). Most of the community trials with DEC standard 12 days-courses (Wijers and Kaleli 1984; Biswas et al. 1989) and repeated (weekly, monthly or yearly) single-doses have demonstrated marked reduction in MF prevalence and intensity (Laigret et al. 1980). A recent community level study showed that six rounds of annual mass treatment of DEC or IVR could reduce MF prevalence by 86 and 72% and geometric mean intensity (Ramaiah et al. 2002) of MF by 91 and 84%, respectively.

1.6.3 Morbidity Control

Studies on the role of bacterial and fungal infections in triggering ADL (acute attacks of ADL) episodes have shown the need for the management of morbidity due to lymphatic filariasis (Dreyer et al. 2000; Addiss et al. 1994; Suma et al. 2002). Simple hygienic measures supplemented with antibiotics can have profound effect

in preventing debilitating and damaging episodes of ADL (Addiss et al. 1994; Olszewski 1996) and to halt or even to reverse the lymphedema and elephantiasis (Partono 1984).

1.7 Socioeconomic Burden of Lymphatic Filariasis

The WHO has developed standardized comparative risk assessment methods for estimating aggregate disease burdens attributable to different risk factors. Changing socioeconomic conditions and physiologic and behavioural adaptations will also affect the vulnerability of populations (McMichael and Githeko 2001; Woodward et al. 1998). LF, a disease which is globally distributed, is a severe social and economic impediment to those infected and is recognized by WHO as one of the most disabling diseases, given 1.3 billion people remain at risk with 120 million infected with some 40 million demonstrating gross pathology.

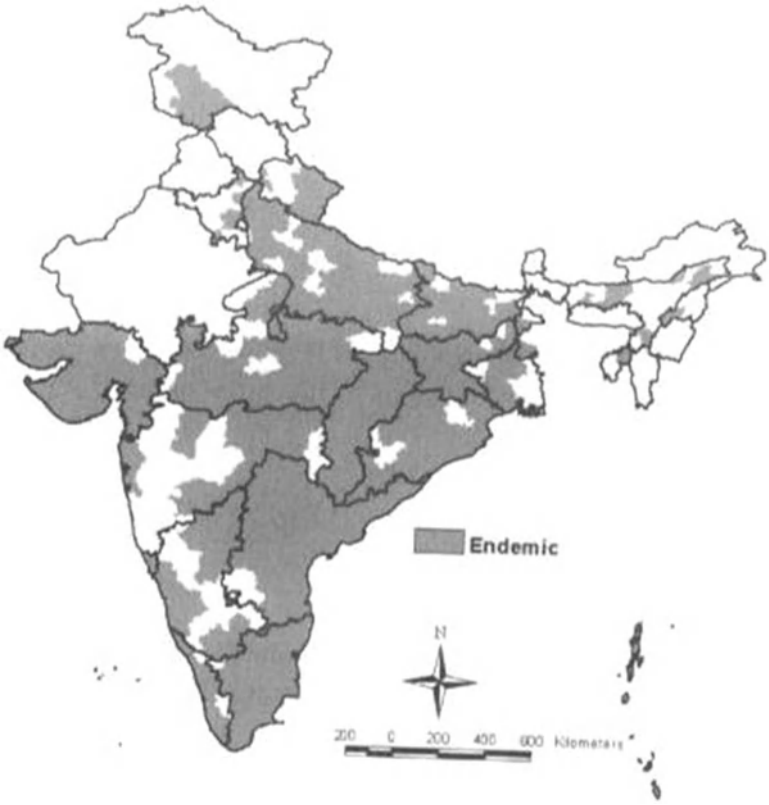
The role of LF in contributing to household poverty has been gathered on a limited scale in Northern Ghana, Southern India and Haiti (Gyapong et al. 1996; Coreil et al. 1998; Ramaiah et al. 1998, 1999; Nanda and Krishnamoorthy 2003). In the Philippines, there is an apparent association between endemicity and poverty at the provincial level. Dunn (1979) observed that the interactions between sociocultural factors and control had largely been ignored, and that less efforts to bridge the gap between biomedical knowledge and indigenous perceptions of disease had been attempted. Among the 80 countries known to be endemic for LF, sociocultural information is available for only 11: Brazil, French Polynesia, Ghana, India, Kenya, Malaysia, Nigeria, Papua New Guinea, the Philippines, Thailand and the United Republic of Tanzania.

1.8 Lymphatic Filariasis in India

Filariasis has been a major public health problem in India. The disease was recorded in India as early as sixth century BC by the famous Indian physician, Susruta in his book "Susruta Samhita" (Bhaskar et al. 2000). The description of the signs and symptoms of this disease by Madhavakara (seventh century AD) in his treatise Madhava Nidhana (Chap. XXXIX) holds good even today. More recently Clarke in 1709 called elephantiasis of the legs in Cochin, South India, "Malabar legs" (Menon and Ramamurti 1940). Lewis, in India, discovered microfilaria in the peripheral blood (Lewis 1872).

Lymphatic filariasis remains a significant health problem in India. Approximately 45% of India's one billion population live in known endemic areas (WHO 2000) and 48 million are infected (Michael et al. 1996), accounting for 40% of the global LF burden. Although the disease severely undermines the socioeconomic progress of the affected communities (Ramaiah et al. 2000), until recently, the control of LF, let alone its elimination, has received little attention in India.

The estimates in 2001 indicate that about 473 million people have been exposed to the risk of bancroftian infection and of these about 125 million live in urban areas and about 348 million in rural areas. About 31 million people are estimated to be harbouring microfilaria (MF) and over 23 million suffer from filarial disease manifestations. However, three relatively less-developed states (Uttar Pradesh, Bihar and Andhra Pradesh) account for 52% of the endemic population and 62% of the infected population (Das et al. 2001). The state of Bihar has the highest endemicity (over 17%) followed by Kerala (15.7%) and Uttar Pradesh (14.6%). Andhra Pradesh and Tamil Nadu have about 10% endemicity. Goa showed the lowest endemicity (less than 1%) followed by Lakshadweep (1.8%), Madhya Pradesh (above 3%) and Assam (about 5%). Seven states, namely Andhra Pradesh, Bihar, Kerala, Orissa, Uttar Pradesh, Tamil Nadu and West Bengal, where MDA pilot trials are being undertaken, contribute over 86% of MF carriers and 97% of disease cases in the country (WHO 2005).



Lymphatic filariasis endemicity in India

The NFCP was launched in India in 1955 with the objective of delimiting the problem and to undertake control measures in endemic areas. The manifold increase in filariasis during the last four decades reflects failure of filariasis control programs

(Sabesan et al. 2000). Currently, the NFCP covers a population of about 40 million (7% of the population at risk), restricted to urban areas only (ICMR Bulletin 2002). Presently there may be up to 31 million microfilaraemics, 23 million cases of symptomatic filariasis, and about 473 million individuals potentially at risk of infection in the country. Lymphatic filariasis is a major impediment to socioeconomic development (estimated economic loss of $1 billion per year) and is responsible for immense psychosocial suffering among the affected (ICMR Bulletin 2002). The National Health Policy 2002 aims at elimination of transmission and the prevention of disability due to lymphatic filariasis by the year 2015, through an MDA programme with an annual single dose of DEC citrate tablets. In the past 5 years, several steps have been initiated in India to move from control to elimination of LF. Initially, MDA was introduced in India as a pilot scheme during 1997 in 13 districts from seven states. In 2001, the programme was scaled up to cover 31 districts from the same states. A massive expansion had been planned in 2004 to cover 201 districts from 20 states and union territories. A nationwide elimination programme requires the distribution of DEC to ~82 million households in 300,000 villages and 1,450 urban agglomerations. At the rate of three drug distributors per village (mean population size of 1,500), the programme needs one million drug distributors (Das et al. 2001).

1.8.1 Information Technology Applications for the Control of Lymphatic Filariasis

Opportunities for control of this devastating disease have arisen from the burgeoning computer technology that supported surveillance of epidemics, which would otherwise be tedious, time consuming, and labour intensive. The difficulties in epidemiological surveillance were circumvented with the introduction of information technology for the integration and transfer of information via a network of interconnected computers. User-friendly software and the availability of computer systems at cheap prices have led to facile generation, processing and dissemination of information. Information systems play a vital role in disease control programs, not only in terms of reporting and data analysis but also in forecasting and prioritization of diseases.

1.9 Applications of Geographic Information System

The powerful tools of spatial technology have revolutionized the way epidemiological research is being conducted. Spatial technology is a field of information technology that acquires, manages, interprets, integrates, displays, analyzes and uses datasets focusing on the geographic, temporal and spatial reference. Spatial technology includes a wide array of technologies such as geographic information system (GIS), remote sensing (RS) and global positioning system (GPS) (Rekha Saxena

et al. 2009). Computers were first applied to geography as analytical and display tools during the 1960s (Tobler 1959). GIS emerged as a multidisciplinary field during the 1970s (Steinitz et al. 1976). GIS has emerged as the core of the spatial technology which integrates a wide range of datasets available from different sources including RS and GPS. GIS has also been described as the technology side of a new discipline, geographic information science (Goodchild 1992), which in turn is defined as "research on the generic issues that surround the use of GIS technology, impede its successful implementation, or emerge from an understanding of its potential capabilities". Recently, GIS has emerged as an innovative and important component of many projects in public health and epidemiology. Recently GIS has been used in the surveillance and monitoring of vector-borne diseases (Beck et al. 1994). GIS tools have contributed immensely in understanding the epidemiological processes of malaria, filariasis, dengue, West Nile virus, to name a few, and thus GIS is now widely used for research and decision making.

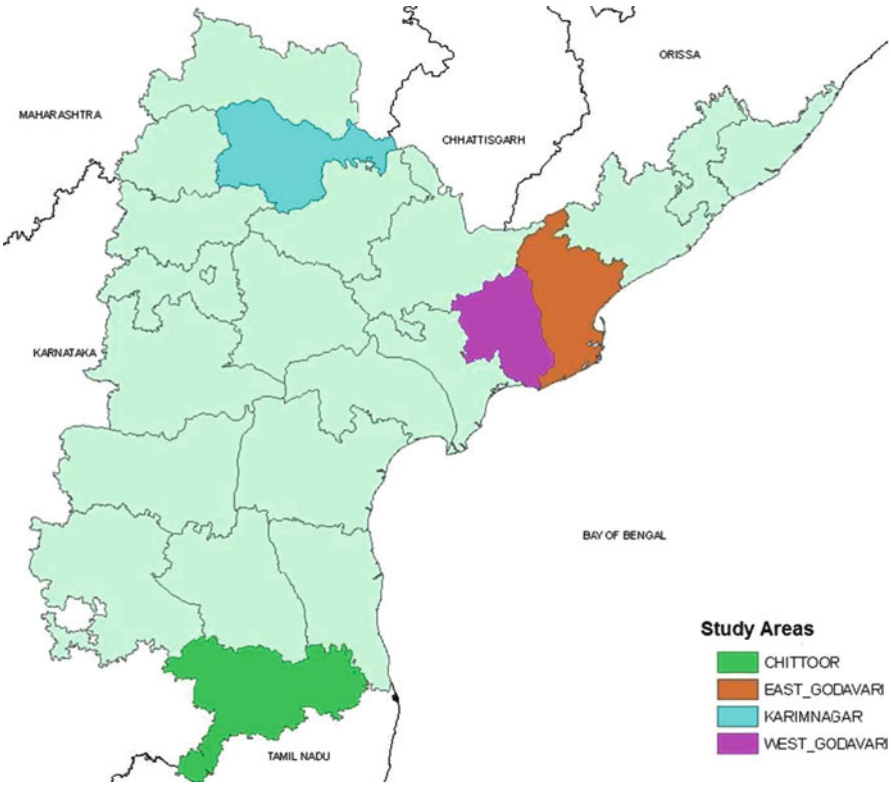
In this chapter, we have applied the spatial analytic methods to epidemiology and conclude by examining the future for technological changes and what these changes mean for control of emerging infectious diseases like lymphatic filariasis. We make an attempt to bridge the gaps between geographic information science (GIS) and public health. The objective of this study is to explore the impact of the intervention coverage and the adherence to the intervention on filariasis health outcomes, through the development of GIS maps based on the entomological and epidemiological data collected in a community-based survey in various districts of Andhra Pradesh, India.

2 Filaria Monitoring Visualization System: A GIS-Based Application

2.1 Study Locations

The study was undertaken in 120 villages from Karimnagar, Chittoor (30 villages from each district), 45 villages from East Godavari and 15 villages from West Godavari district of Andhra Pradesh, India during 2004–2007. Karimnagar district is a part of Telangana and lies on the Northern part of Andhra Pradesh approximately between the 18°25′48″N and 79°9′0″E. Chittoor district is a part of Rayalaseema and lies in the extreme South of the state approximately between 12°37′–14°8′ North latitudes and 78°3′–79°55′ East longitudes. The East Godavari district is located in the North coastal part of Andhra Pradesh. The district is located between Northern latitudes of 16°30′ and 18°20′ and the Eastern longitudes of 81°30′ and 82°30′. Similarly, West Godavari district is a part of the Godavari delta in Andhra Pradesh. It lies between 16°15′ and 17°30′ Northern latitudes and 80°55′ and 81°55′ Eastern longitudes. The climate is characterized by a summer (46–20°C), winter (32–11°C) and monsoon (June–December). The South West monsoon plays

a major role in Andhra Pradesh. The North East monsoon is responsible for about one third of the total rainfall in Andhra Pradesh.



Map showing study areas (districts) in Andhra Pradesh

2.2 Study Design

For this study, 120 villages were selected by stratified random sampling from the four districts of Andhra Pradesh. Before investigations, the local authorities and the residents of the study villages were informed about the proposed study for their authorization. Individuals from all households involved were offered filarial treatment during the period of the study. Before collecting blood samples all individuals who participated were interviewed, using a structured questionnaire, on their knowledge, attitude and behaviour in relation to filariasis. A record of age, sex, occupation, educational status, socioeconomic status details and mosquito avoidance adopted in the household, etc. was kept. The households were selected by stratified random sampling methodology.

The survey was carried out from the endemic villages to include diseased individuals in the study. Twenty-four thousand blood smears were collected during the study period (2004–2007) from the households from Karimnagar, Chittoor, East and West Godavari districts of Andhra Pradesh. Each selected household was considered as a sampling unit, and all individuals present at the time of survey were registered for screening of microfilaraemia and disease manifestations through house-to-house visits. About 20 µl of blood was collected, between the 20:00 and 23.00 h, from each person by using finger prick method for a prepared smear on clean glass slides (Sasa 1976). The parasites were examined and counted via microscopy. The study received ethical clearance from the Ethics Committee.

Indoor-resting mosquitoes were collected with the help of mechanical aspirators (Hausherr's Machine Works, NJ, USA) during 06:00–09:00 h from the study areas during the study period (2004–2007). Only female *C. quinquefasciatus* mosquitoes, the principal vectors of Bancroftian filariasis, were identified by using the key developed by Reuben et al. (2001) and subjected to dissection. The vector abundance is expressed as the number of female *C. quinquefasciatus* mosquitoes collected per man per hour (PMH). In order to assess the transmission levels of the disease, *C. quinquefasciatus* mosquitoes have been dissected to identify the stage of the microfilaria, using the key developed by Nelson (1959) and Yen et al. (1982) and the infection rate was calculated by the presence of any stage of microfilaria and infectivity rates were recorded based on the presence of third stage of microfilaria (MF) only.

$$\text{Infection rate } (\%) = \frac{\text{No. positive for } L_1, L_2, L_3 \text{ stage}}{\text{No. of mosquitoes dissected}} \times 100$$

$$\text{Infectivity rate } (\%) = \frac{\text{No. of mosquitoes +ve for } L_3 \text{ stage}}{\text{No. of mosquitoes dissected}} \times 100$$

$$\text{Microfilaria rate } (\%) = \frac{\text{Positive blood smears}}{\text{Total blood smears examined}} \times 100$$

2.3 Collection of GPS data

GPS is a system of 24 satellites that allows the coordinates of any point on or near earth's surface to be measured with extremely high precision (Boulos et al. 2001). GPS is a satellite-based navigation system made up of a network of 24 satellites placed into orbit, called GARMIN satellite system. The interception of a minimum of three satellite signals allows the GPS receiver to calculate its position on the earth with respect to latitude and longitude. A minimum of four satellite signals is required to include altitude calculations. Garmin, a GPS handheld receiver has been used in

this study. GPS locations (latitude, longitude, altitude) of the study villages were registered as Waypoints, throughout the field survey from the four districts of Andhra Pradesh.

2.4 Data Preparation

The data pertaining to filariasis and mosquitoes were collected from the ground survey and subsequently processed and analyzed through various methods. The resultant information was attached to spatial data for visualization and query purposes in the developed application. The development of a filarial monitoring visualization system (FMVS) utilized various datasets as given below.

- Survey of India (SOI) Village boundary maps
- GPS data of Surveyed villages
- Filariasis survey results (epidemiology data)

2.5 Data Processing and Analysis

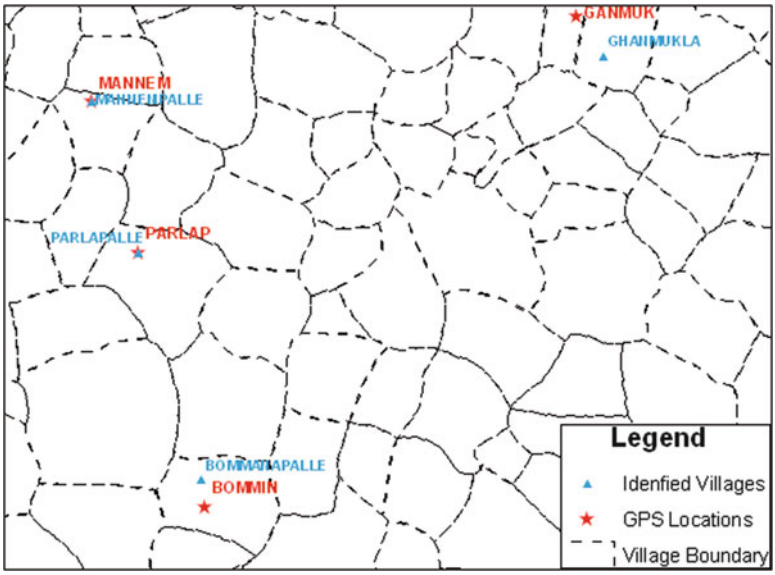
The collected epidemiology and entomology data have been classified into low, medium and high regions. The parameters used for this classification were per man hour density (PMHD), microfilaria (MF) rate, infection rate and infectivity rate.

2.5.1 Classification of Parameters

Parameters	Low	Medium	High
PMHD	0–10	10–40	>40
Infection rate	0–5	5.1–10	>10
Infectivity rate	0–0.2	0.2–1	>1
MF rate	1–5	5–10	>10

2.5.2 Spatial Data Preparation

The basic spatial data for this application were village boundaries that were made available with NRSA. GPS data were used for identifying the surveyed villages. Once the villages were identified, the data were linked as a common field between spatial and filariasis databases in a specific format suitable for application development.



2.6 Tools and Technology Used

The following tools and technologies were adapted for developing the application.

S. no.	Component	Product used
1	Operating system	Microsoft Windows
2	GIS engine	ArcGIS Engine 9.2
3	Programming language	Microsoft .net
4	Database system	MS Access

2.7 Development of FMVS Application

Customization of a GIS application is the process of leveraging the available functionalities in a desired manner from software development kits (SDKs). ArcGIS Engine is one such kit of GIS functionalities which can be accessed through any programming language such as C++, VB, .Net, and Java. For the current application, .Net was used to incorporate various functionalities such as data accessing, data visualization and thematic representation based on attributes. Description of various modules is given below and the architecture of the application is represented in Fig. 7.3.

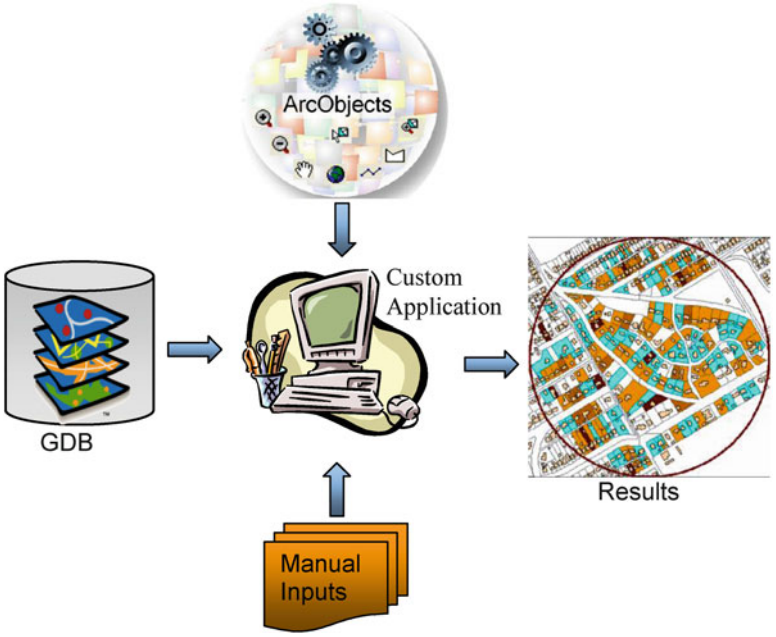


Fig. 7.3 Architecture of the filaria monitoring visualization system (FMVS)

2.8 Usage and Guidelines

2.8.1 Data Accessing Module

The current application data are stored in ESRI Personal Geodatabase (GDB) format which contains spatial layers such as village boundaries and GPS locations of each of four study area districts with the Survey of India (SOI) standard of naming conventions. The data accessing module is invoked to display in map window(s), the selected district data and subsequently the user can choose the data through the combo box provided in the interface.

2.8.2 Visualization Module

The data displayed in the map window can be viewed at various levels by the navigation functionalities provided in the tool bar such as panning, zooming and full extent. A simple data query tool is also available to view the attributes of a selected village.

2.8.3 Thematic Representation Module

There are four parameters (infection rate, infectivity rate, PMH and MF rate) that were divided into three intensities such as low, medium and high. To represent these intensities, a unique value rendering functionality is used to represent in different colours the corresponding attributes of each village. Villages having no data will have no colour. A unique feature of this application is having four geometrically synchronized map windows, which are used to present each parameter in different windows of the same study area. A location map is provided to display the study area location in the state map.

2.9 User Interface and Description

Users of this tool have access to various modules through a graphic user interface (GUI). FMVS has two windows, the splash screen and the main window. When the application is started the splash screen gets displayed, on pressing the proceed button the progress bar proceeds as the data get loaded into the application.

After the data are loaded the splash screen automatically gets closed, on opening the main map window as shown in Fig. 7.4. The main window consists of four map windows to represent four parameters of the same district at the same time and a location map of the district. A drop-down box is provided to choose any one of the districts, which will be reflected in both map windows and location map areas (Figs. 7.5, 7.6, 7.7, and 7.8).

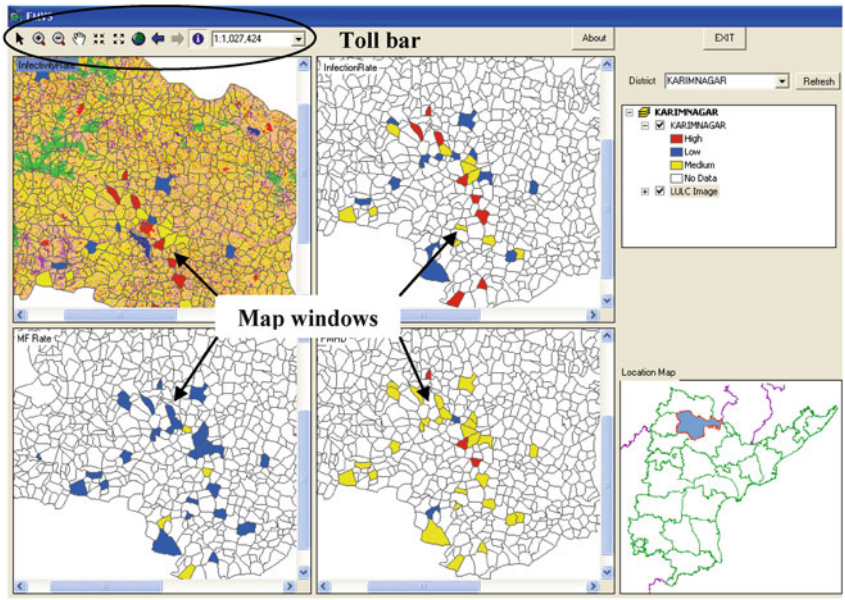


Fig. 7.4 Main window displaying Karimnagar district

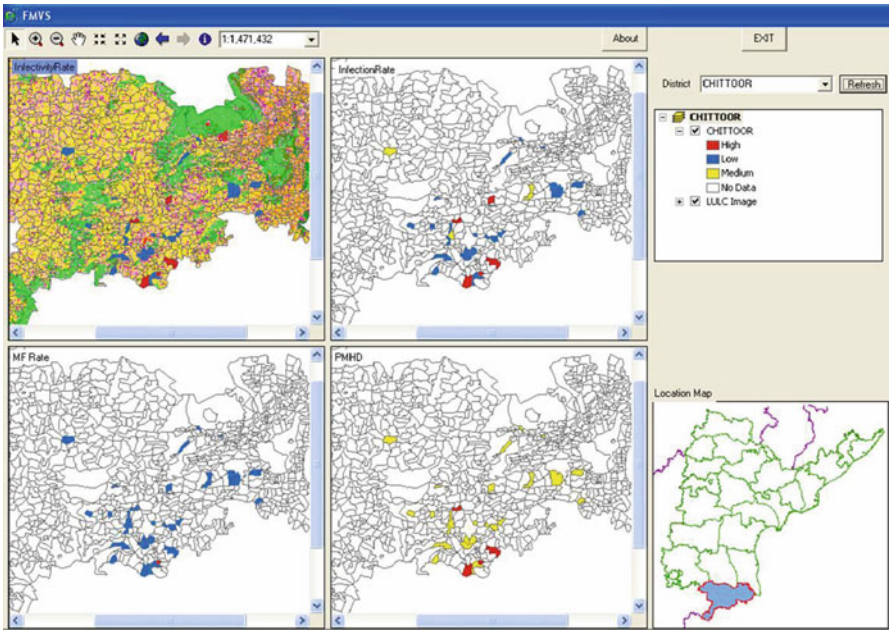


Fig. 7.5 Spatial map showing the village level prevalence of filariasis (infectivity, infection, MF rate and PMH) in Chittoor district of Andhra Pradesh

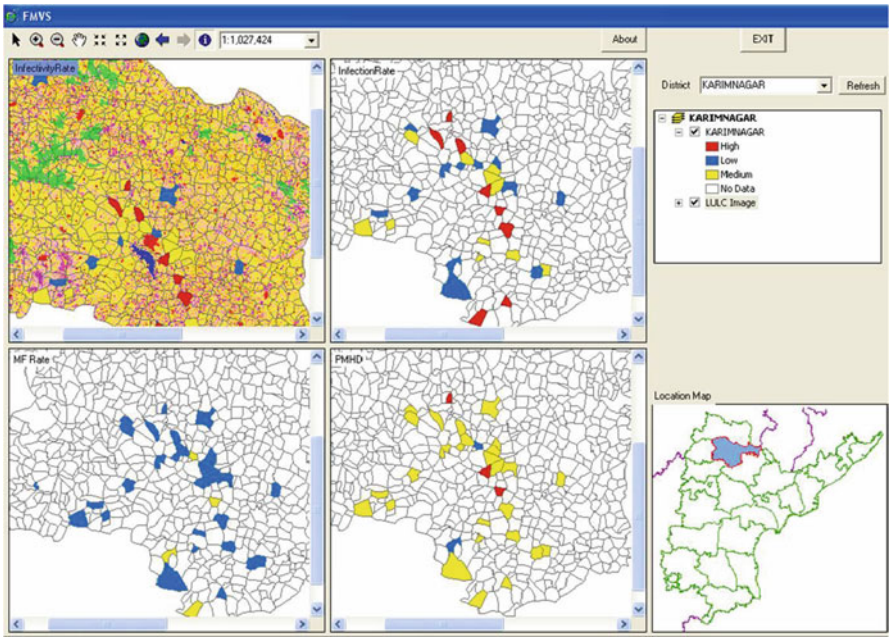


Fig. 7.6 Spatial map showing the village level prevalence of filariasis (infectivity, infection, MF rate and PMH) in Karimnagar district of Andhra Pradesh

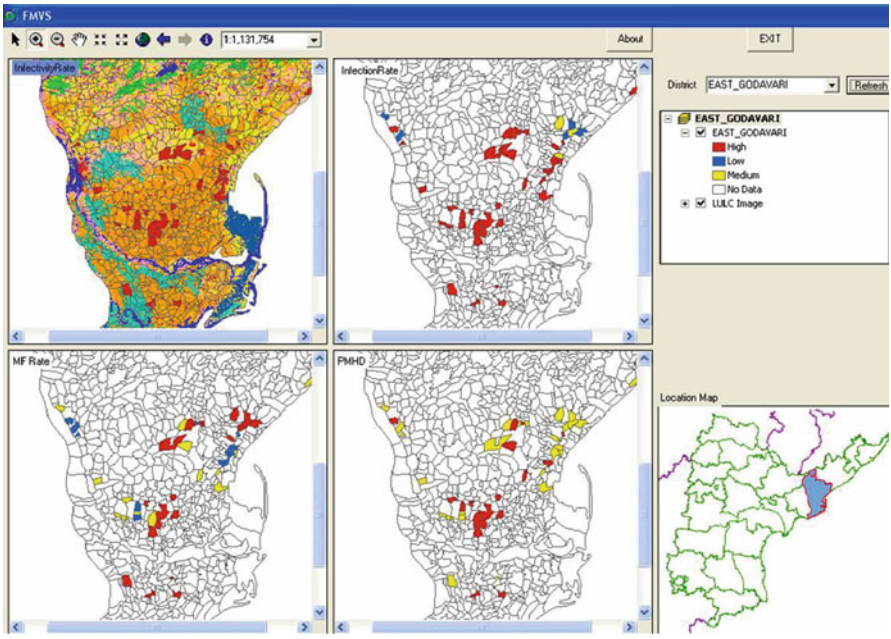


Fig. 7.7 Spatial map showing the village level prevalence of filariasis (infectivity, infection, MF rate and PMH) in East Godavari district of Andhra Pradesh

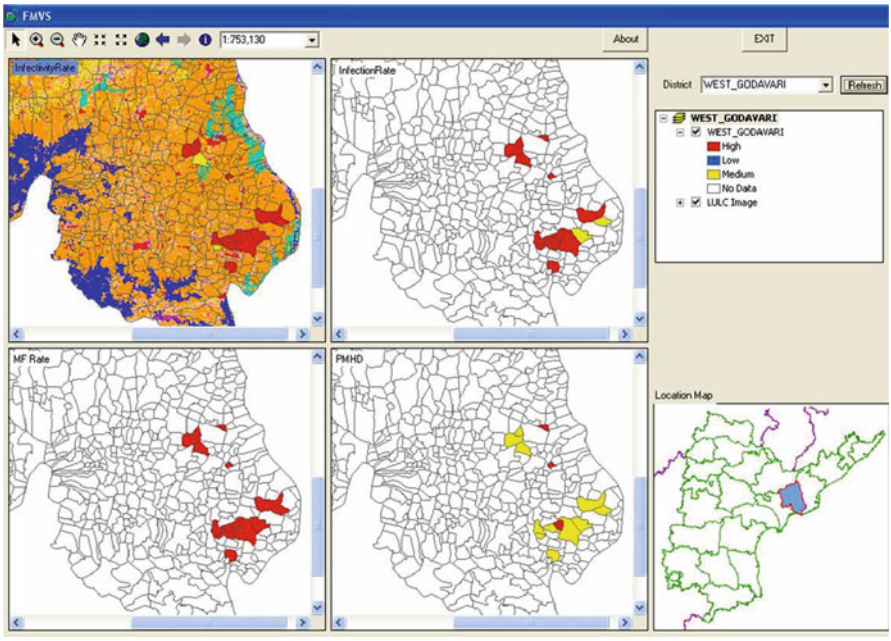


Fig. 7.8 Spatial map showing the village level prevalence of filariasis (infectivity, infection, MF rate and PMH) in West Godavari district of Andhra Pradesh

3 Conclusions

In India, an estimated 450 million people living in 257 districts across 18 states and Union territories are at risk for filarial infection. However, three relatively less-developed states (Uttar Pradesh, Bihar and Andhra Pradesh) alone account for 52% of the endemic population and 62% of the infected population (Das et al. 2001). National Health Policy 2002 aims at elimination of transmission and the prevention of disability due to lymphatic filariasis by the year 2015, through an MDA programme with an annual single dose of DEC citrate tablets. Sixteen out of 23 districts of Andhra Pradesh are under the grip of filariasis and 54 million people in the state are under an "MDA" programme with an annual single dose of DEC tablets (NVBDCP 2004). Chittoor, Karimnagar, East and West Godavari are among the worst affected districts in Andhra Pradesh. In an attempt to eradicate filariasis in East Godavari, since 1999–2005, a total of six rounds of MDA programmes were organized covering five million people (AP Annual report 2005).

The risk factors for infection and disease due to *W. bancrofti* have been difficult to characterize because of the complex life cycle of this mosquito-borne helminth and because of the broad range of clinical signs and symptoms attributable to this nematode. A longitudinal survey has been conducted in four districts (Chittoor, Karimnagar, East and West Godavari) of Andhra Pradesh to evaluate the endemicity of lymphatic filariasis. The results from the epidemiological survey indicate that filariasis is endemic in the Chittoor, Karimnagar, East and West Godavari districts of Andhra Pradesh. The mean prevalence rate for microfilaraemia in Chittoor was 0.883%, Karimnagar 2.067%, East Godavari 10.982% and West Godavari 22.713%. From the WHO classification, most of the villages from East and West Godavari districts were seen to be hyperendemic (>10%). Similarly, low (<5%) and medium (<10%) endemicity was reported, respectively, from the Karimnagar and Chittoor districts of Andhra Pradesh.

The filaria monitoring and visualization system developed on a GIS platform could be employed for spatial delimitation of filariasis, particularly to identify risk areas, more precisely. *W. bancrofti* transmission is determined by several variables, and hence it is possible to characterize areas with this monitoring system, where risk of transmission can be determined on a micro and macro-scale. Environmental conditions are widely conducive to transmission efficiency; human factors are also key determinants contributing to the local occurrence of filariasis. The human factors exhibiting major influence include population density, movement, economic status, occupation, literacy level and health-seeking behaviour (Jeevan Sherchand et al. 2003; Galvez Tan 2003). Similarly, vector abundance may vary widely depending on geo-physical and human-associated factors, but the vector survival and capacity for parasite development (vectorial capacity) and the transmission of infection are greatly determined by various ecological factors. The prevalence and intensity of filariasis in human population is directly related to the parameters such as vector infection, infectivity and biting density of infective vector populations in the endemic areas, which in turn are influenced by variations in climate.

This filaria monitoring and visualization system will help the end user to quickly assess the intensity of the disease based on the information provided by the visualization system. The health official can take appropriate control measures in accordance with the intensity of the parameter displayed by the FMVS. This will enable public health officials to initiate correct and efficient integrated control measures. The FMVS is easily operable and can be transported on all working platforms and thus this integration of data will be of immense help to the end user.

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Chapter 8
The Global Burden of Severe Falciparum Malaria: An Immunological and Genetic Perspective on Pathogenesis

Douglas J. Perkins, Tom Were, Samuel Anyona, James B. Hittner, Prakasha Kempaiah, Gregory C. Davenport, and John Michael Ong'echa

1 Introduction

Plasmodium falciparum malaria is a leading global cause of morbidity and mortality of infectious disease origin. Here, we focus largely on *P. falciparum* malaria in sub-Saharan Africa since this geographic region bears the greatest disease burden, resulting in exceedingly high rates of morbidity and mortality. The life cycle, etiology, and epidemiology of *P. falciparum* are also presented. In addition, we provide a detailed discussion of the pathophysiology of severe, life-threatening complications

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of falciparum malaria such as cerebral malaria (CM), severe malarial anemia (SMA), hyperparasitemia, hypoglycemia, hyperlactatemia, electrolyte and fluid imbalances, renal dysfunction, metabolic acidosis, and respiratory distress (RD) (Marsh et al. 1995; WHO 2000). A comprehensive overview on the role of cytokines, chemokines, growth factors, effector molecules, and antibodies is also presented in the context of innate and acquired immunity. Since susceptibility to falciparum malaria and the clinical outcomes that result following an infection are conditioned by genetic variation, a detailed description of different genetic studies is presented, including the candidate gene approach, linkage disequilibrium (LD), and genome-wide association (GWA) studies. Lastly, we provide a detailed description of the statistical modeling we have employed to examine the association between malaria disease outcomes and host genetic and immunological factors.

2 Etiology of Malaria

Human malaria is caused by the unicellular obligate intracellular protozoan parasites of the genus *Plasmodium*. Four species of malaria parasites infect humans: *P. falciparum*, *P. ovale*, *P. malariae*, and *P. vivax*. Of these species, *P. falciparum* is the most virulent of the human malaria parasites and is responsible for the bulk of the malaria-related morbidity and mortality. *P. falciparum* accounts for 91% of malaria cases worldwide of which 86%, 9%, and 3%, respectively, occur in the African region, South East Asia, and Mediterranean region (WHO and UNICEF 2008). Consistent with geographic distribution of the largest amount of morbidity in sub-Saharan Africa, greater than 90% of the *P. falciparum*-attributable malaria deaths occur in the African region, while approximately 4% of the additional mortality is represented in South East Asia, with the remaining proportion (~4%) in Eastern Mediterranean regions (WHO and UNICEF 2008). Malaria due to *P. vivax*, *P. ovale,* and *P. malariae* is less severe and accounts for fewer than 10% of the malaria cases worldwide (WHO and UNICEF 2008).

3 Life Cycle of *P. falciparum*

The life cycle of *P. falciparum* malaria parasites involves two phases: an endogenous asexual stage in humans and an exogenous sexual stage in the mosquito (Fig. 8.1). Human infection is initiated by a bite of an infected female *Anopheles* mosquito vector during a blood meal. The mosquito injects a small number of sporozoites (~15–40) into the bloodstream and this is sufficient to establish liver infection (Frischknecht et al. 2004). The sporozoites survive in the bloodstream for up to 4 h, unless destroyed by circulating phagocytes. The micronemal proteins, such as the thrombospondin-related anonymous protein, mediate sporozoite invasion of hepatocytes, where they initiate an asymptomatic pre-erythrocytic schizogony or

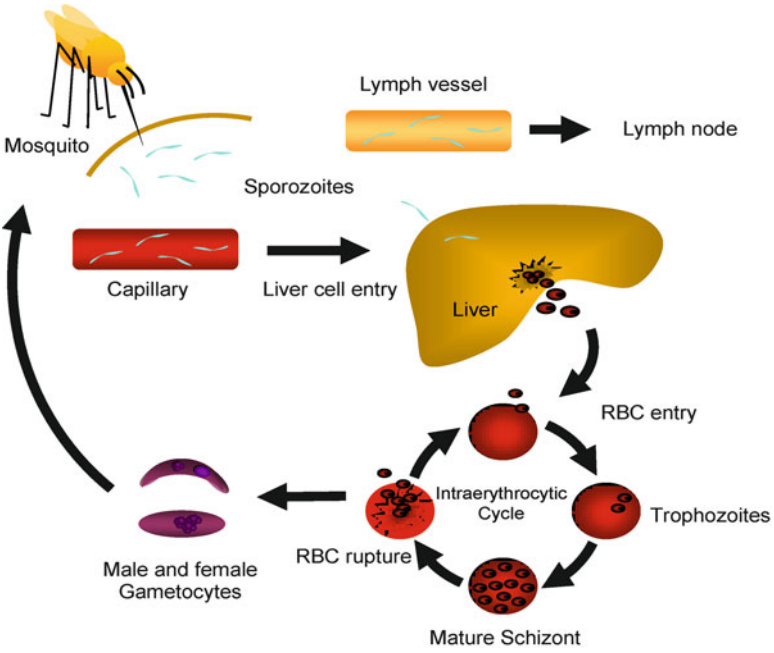

Fig. 8.1 The life cycle of *Plasmodium falciparum*. *Source:* Redrawn from figure with permission from Elsevier

merogony (Yuda and Ishino 2004). In the liver, the sporozoites enlarge and undergo repeated nuclear division into several daughter nuclei (pre-erythrocytic or exoerythrocytic schizonts or meronts). As the schizonts continue development, the hepatocyte is distended by the enlarging schizonts and the nucleus is pushed towards the periphery. Unlike during erythrocytic schizogony (discussed below), there is no formation of malarial pigment hemozoin (Hz) within the liver schizonts. In approximately 5–15 days, the schizont matures and ruptures, releasing thousands of small, round merozoites into the blood stream where they then proceed to infect red blood cells (RBCs).

The invasion of RBCs is a rapid process that is completed within approximately 30 s. The receptor that facilitates the invasion of merozoites into RBCs is a sialoglycoprotein on the RBC membrane (i.e., glycophorin) (Pasvol 2003). In addition, sialic acid-independent pathways are also important for invasion (Pasvol 2003). The merozoites enter RBCs by endocytosis and are then enclosed by the erythrocyte membrane into a vacuole (i.e., parasitophorous vacuole). Merozoites initiate erythrocytic schizogony in which they produce an average of 16 erythrocytic merozoites per schizont every 48 h. The merozoites feed on hemoglobin (Hb) inside the RBCs and hydrolyze the heme from Hb into Hz through the action of plasmepsin and falcipain enzymes in the food vacuole of the parasite (Banerjee et al. 2002). Hz is released from the RBCs when the RBC ruptures and it is then subsequently taken up by cells of the reticuloendothelial system including circulating monocytes and

neutrophils. As the merozoites mature and develop into schizonts, the RBCs rupture and release more merozoites into the bloodstream that then proceed to infect new RBCs. This cycle continues with exponential phases of parasitic growth until the human host succumbs to high levels of parasitemia and dies, therapeutic treatments are implemented to inhibit this process, or host immune protective mechanisms bring the parasitemia under control. Although the pre-erythocytic stage represents the first encounter of the human host with the parasite, the erythrocytic stage, characterized by the cyclic rupture of the mature schizonts and concomitant release of antigens and waste products, is responsible for the clinical symptoms and development of immunity to malaria (Hviid 2005).

After several generations of reproduction, a fraction of the merozoites develop into female and male gametocytes: macrogametocytes and microgametocytes, respectively. The gametocytes are taken up by the female mosquito during feeding on an infected human host and then form the female macrogametes and male microgametes. The microgametes fertilize the macrogametes and produce a zygote, which develops into an ookinete that penetrates the epithelial lining of the mosquito gut and forms an oocyst (Smith et al. 2000). The oocyst matures and ruptures, releasing new sporozoites that then migrate to the mosquito salivary glands from where they are injected into a human host during the mosquito's subsequent blood meal (Sinnis 1996).

4 Epidemiology of *P. falciparum* Malaria

Although malaria was once prevalent throughout most of the world, it is currently endemic in the tropical regions with extensions into the subtropical regions of Asia, Africa, and South and Central America (Fig. 8.2). However, approximately half of the world's population (3.3 billion people) is at risk for malaria in more than 100 countries (Snow et al. 2005). In addition, the endemicity of malaria varies with climatic conditions from country to country, and within the differing microclimates within the countries themselves (Snow et al. 2005). An estimated 300–660 million clinical cases and one to two million deaths are attributable to *P. falciparum* malaria annually around the globe (Snow et al. 2005).

Immune-naïve individuals, such as children below 5 years of age and pregnant women, suffer the highest malaria burden (WHO 2000; Snow et al. 2005). An estimated 70% and 25% of the world malaria disease incidences and deaths are concentrated in sub-Saharan Africa and South East Asia, respectively (Snow et al. 2005). The higher malaria burden in Africa is due to the predominance of *P. falciparum* endemicity, the most pathogenic human malaria parasite, and the presence of *Anopheles gambiae* mosquitoes, the most widespread and efficient malaria vector in Africa (Coetzee et al. 2000). Additionally, *A. gambiae* has become increasingly resistant to current insecticides (Yawson et al. 2004). The development and spread of multidrug resistant *P. falciparum* infections in developing nations has added to the worsening malaria burden in Africa (Plowe et al. 2007). The current situation is

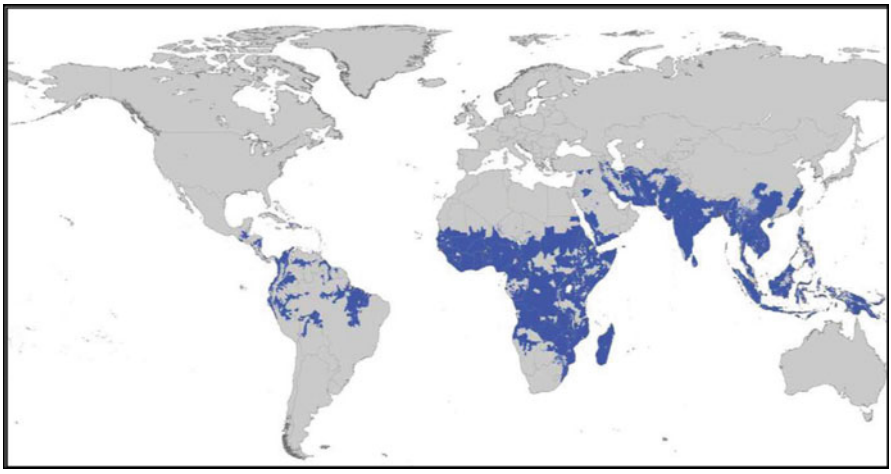

Fig. 8.2 Distribution of reported malaria cases. *Source*: Snow et al. (2005). The *blue areas* show global *P. falciparum* endemic regions, while the *gray shaded areas* indicate non-*P. falciparum* endemic areas. Reprinted with Permission, Nature Publishing Group

further aggravated by the lack of effective health-care facilities, particularly in rural sub-Saharan Africa, where populations are subject to the highest malaria burden (Agyepong and Kangeya-Kayonda 2004).

P. falciparum malaria directly or through synergy with other infections and illnesses causes more than one million deaths each year, primarily in young children. The endemicity patterns of malaria transmission and clinical outcomes of the disease vary widely across regions and even within countries. This diversity is largely due to variation in malaria parasites and mosquito vectors, ecological conditions, and socioeconomic factors. Malaria is also a major cause of anemia in children and pregnant women, and is responsible for low birth weight infants, premature birth, and infant mortality (Murphy and Breman 2001). In endemic African countries, malaria accounts for 25–35% of all outpatient visits, 20–45% of the hospital admissions, and 15–35% of the hospital deaths, imposing an overwhelming burden on the already fragile health-care systems of emerging economies (Snow et al. 2005).

5 Pathogenesis of *P. falciparum* Malaria

The clinical spectrum of *P. falciparum* malaria encompasses a wide range of pathophysiological derangements that can involve multiple organs and systemic disorders. The spectrum of malaria manifestations varies from asymptomatic infections to the classic symptoms of malaria (e.g., fever, chills, sweating, headache, and muscle aches). At the far end of the clinical spectrum are severe, life-threatening complications such as CM, SMA, hyperparasitemia, hypoglycemia, hyperlactatemia, electrolyte and fluid imbalances, renal dysfunction, metabolic acidosis,

and RD (Marsh et al. 1995; WHO 2000). The pathophysiology of malaria, however, is complex, multifactorial, and only partially understood. The development of a pathogenic versus protective immunological response to malaria is determined, to a large extent, by host and parasite-related factors including age of first infection, prior acquisition of immunity, parasite virulence, parasite multiplication rate, antigenic variation within the parasites, endemicity patterns, and polymorphic variability within the human host and malaria parasite (Abdalla and Pasvol 2004).

6 Primary *P. falciparum* Infection

The clinical presentation of a *P. falciparum* infection occurs 7–10 days after parasitic inoculation and can include fever, sweating, chills, headache, muscle ache, and presence of parasites in the peripheral circulation (Perkins et al. 1997). The febrile response results from the release of pyrogenic cytokines, including tumor necrosis factor (TNF)-α and interleukin (IL)-1β that stimulate the hypothalamic region of the brain to induce a febrile response (McGuire et al. 1998; Mordmuller et al. 1997).

7 *P. falciparum* Infection Resulting in Severe Malaria

If the primary infection is not properly controlled through either pharmacological intervention(s) or successful host-mediated immunity, the primary infection can progress to severe, life-threatening complications in individuals infected with *P. falciparum* infections, particularly in those that lack malarial immunity. The severe manifestations of falciparum malaria are discussed below.

7.1 Cerebral Malaria

CM commonly occurs in areas of low-to-moderate endemicity with seasonal variation and primarily affects older children, adolescents, and adults with low levels of acquired immunity to malaria (Snow et al. 1997). The mechanisms responsible for the development of CM result from impairments in cerebral perfusion, local alterations of the blood–brain barrier, and adherence and sequestration of parasitized red blood cells (*p*RBCs), as well as non-parasitized RBCs to microvascular endothelial cells (Newton et al. 2000). Sequestration of the *p*RBCs and non-parasitized RBCs in the cerebral vasculature is mediated by *P. falciparum* erythrocyte membrane protein (*Pf*EMP)-1 expressed on the surface of *p*RBC, and binding to ligands such as ICAM-1 which are typically up-regulated on the endothelial lining of the cerebral venules in response to pro-inflammatory stimuli (Newton et al. 2000). The process of sequestration is enhanced when adherent *p*RBCs bind to other infected RBCs

(auto-agglutination), non-infected RBCs (rosetting), and/or platelets (platelet-mediated clumping) (Idro et al. 2005). There is also evidence that sequestration of T cells, monocytes, and platelets occurs in the cerebral vasculature in patients with CM (Grau et al. 2003; Renia et al. 2006). Taken together, CM is a severe disease manifestation that is mediated, at least in part, by the inflammatory cascade in the human host.

More recent studies illustrate that CM is an important cause of neurocognitive derangements (Carter et al. 2003). One investigation has shown that CM is associated with persistent multiple neurocognitive impairments and increased risk of mortality in the first year following discharge from the hospital (Idro et al. 2006). Another study has shown that there is a higher incidence of sequelar epilepsy in children who previously experienced CM (Ngoungou et al. 2006), suggesting that CM may negatively impact on the cognitive development of children in malaria endemic areas. Although documentation for the long-term effects of neurocognitive impairments remains largely undefined, a number of ongoing studies should provide definitive evidence (in the near future) about lasting effects of CM-associated neurocognitive impairments.

7.2 Severe Malarial Anemia

Anemia is defined as a reduction in Hb levels in relation to age and physiological status of the individual within a particular geographic context (Murray et al. 1996). In western countries, anemia is defined by an Hb concentration <12.0 g/dL, while in developing countries the standard definition of anemia for children <5 years of age is Hb < 11.0 g/dL (Murray et al. 1996). The World Health Organization (WHO) defines SMA as a Hb concentration <5.0 g/dL (or a hematocrit <15.0%) in the presence of a parasitemia (WHO 2000).

SMA is a major public health problem in many developing countries where it contributes 3–46% of the inpatient pediatric fatalities in referral care facilities (English et al. 2004). Despite efforts aimed at ameliorating the anemia burden, SMA remains an important childhood health burden in sub-Saharan Africa (Brabin et al. 2001a). Previous studies demonstrated that the annual rate of hospital presentation with SMA was 7.6/1,000 in children 0–4 years of age, with a case fatality of 9.7% in endemic areas of Africa (Snow et al. 1999). Other studies in Tanzanian children illustrate that the risk for SMA peaks at 1 year of age in high transmission areas and at 2 years of age in moderate and low transmission intensities, and then subsequently decreases with increasing age (Reyburn et al. 2005). In western Kenya, SMA is highest in children below 3 years of age with peak prevalence in the 7–24 month age group (Bloland et al. 1999; McElroy et al. 1999). Multicenter studies indicate that SMA affects 7.5–34% of the African children with malaria with an overall prevalence of 21.2% (Taylor et al. 2006a). Other studies showed that SMA is associated with an overall case fatality rate of 8.4% among children with severe malaria (Taylor et al. 2006a).

SMA is also an important public health problem among pregnant women in the endemic countries of Africa (Brabin et al. 2001b). Other vulnerable groups include

adolescents and school-age children (Murray et al. 1996). Adults and the elderly may also be at risk, especially where there is inadequate food intake, nutritional deficiencies, frequent parasitic infestations, and/or co-infections with human immunodeficiency virus (HIV) and bacteremia (Bronzan et al. 2007; Otieno et al. 2006). Development of SMA is regulated by a number hemoglobinopathies such as sickle cell trait and thalassaemias, and red cell enzymopathies such as glucose-6-phosphate dehydrogenase (G6PD) deficiency, all of which are represented at high prevalence in malaria endemic regions of Africa (Abdalla and Pasvol 2004).

The etiology of SMA is complex and multifactorial and often includes direct and indirect destruction of infected and uninfected erythrocytes (Ekvall et al. 2001). The basic mechanisms underlying the markedly reduced Hb levels that characterize SMA involve erythrophagocytosis, dyserythropoiesis, and suppression of erythropoiesis (Abdalla and Pasvol 2004). The finding that children with bone marrow suppression are associated with persistent *P. falciparum* infections (Helleberg et al. 2005) suggests that the inflammatory response is likely an important contributing factor governing both the suppression of erythropoiesis and SMA. Other mechanisms of SMA pathogenesis include autoimmune hemolysis of RBCs mediated by IgG antibodies directed against RBC membrane antigens such as band 3 and spectrin (Abdalla and Pasvol 2004).

7.3 Hyperparasitemia

Hyperparasitemia is defined as parasite count greater than 100,000 parasites/μL. Although hyperparasitemia is a criterion for classification of severe malaria recommended by the WHO, the degree of parasitemia is often times not an accurate prognostic indicator of disease severity (Lyke et al. 2003; Ong'echa et al. 2006). To properly account for the overall level of peripheral parasitemia, it is important to express the parasite density according to the number of leukocytes (typically the white blood cell count). During *P. vivax* or *P. ovale* infections, fever tends to be observed at a lower level of parasitemia than is typically witnessed in *P. falciparum* infections; however, hyperparasitemia and associated complications are more common in cases of *P. falciparum* malaria (Hemmer et al. 2006). Hyperparasitemia was first defined by Field et. al., in Peninsular Malaya, based on their observation that parasite counts that reached >100,000 parasites/μL were associated with increased mortality, and when parasitemia exceeded 500,000 parasites/μL, half of the patients died (Field and Niven 1937). A blood transfusion is recommended when the parasite count reaches >200,000 parasites/μL (regardless of the clinical situation) and if the parasite count is >100,000/μL in the presence of additional clinical complications (White 1996). In individuals with little or no background immunity to malaria, hyperparasitemia, in the context of acute disease, is a medical emergency and, if left untreated, may progress to vital organ dysfunction and death (Luxemburger et al. 1995).

While parasitemia does not always predict the degree of disease severity, hyperparasitemia does increase the risk of hypoglycemia, metabolic acidosis, RD, CM,

and SMA, and has some predictive ability for mortality (Marsh et al. 1995; WHO 2000; Beadle et al. 1995; McElroy et al. 1994). In summary, it cannot be assumed that low levels of parasitemia necessarily indicate a mild form of infection, but hyperparasitemia, particularly in children and nonimmune individuals, may lead to hemolysis and enhanced clearance of RBCs, resulting in profound anemia (Phillips et al. 1986; Molyneux et al. 1989).

7.4 Hypoglycemia

Hypoglycemia is a major clinical complication of altered carbohydrate metabolism in individuals with falciparum malaria. As many as 16% of children with moderate or severe malaria present with hypoglycemia (English et al. 1998). Recent studies in Nigerian children illustrate that hypoglycemia is associated with severe malaria and enhanced mortality (Elusiyan et al. 2006). Hypoglycemia is present in approximately 20% of the CM cases (English et al. 1998), and is an important predictor of enhanced mortality in children with severe malaria (Dzeing-Ella et al. 2005). Although the mechanisms underlying the development of hypoglycemia in children are poorly understood, it appears to be the product of a combination of decreased production and/or increased peripheral uptake of glucose, due to increased anaerobic glycolysis (Planche et al. 2005). In adult patients with CM, however, hypoglycemia is associated with increased glucose turnover and quinine-induced hyperinsulinemia (Planche et al. 2005).

7.5 Hyperlactatemia

Hyperlactatemia is a disorder of carbohydrate metabolism associated with severe malaria. Previous studies in Gabonese children showed that hyperlactatemia was present in 16% of the children with severe malaria, and represented an important prognostic indicator of high rates of fatality (Planche et al. 2003). Additional studies in Ghana also demonstrated that high plasma lactate levels "independently" predicted mortality in children with severe malaria, and were associated with deep coma (Planche et al. 2003). Recent studies in Kenyan children demonstrating elevated plasma lactate levels in children with SMA (Casals-Pascual et al. 2006) suggest that increased glycolytic anaerobic production of lactate is a complicating feature of severe malaria syndromes.

Although the mechanisms underlying hyperlactatemia are poorly understood, increased anaerobic glucose metabolism appears important. Enhanced anaerobic glucose metabolism may result from increased microvascular sequestration of *p*RBCs which reduce blood flow to tissues and induce an inflammatory response (Planche et al. 2005). This hypothesis is supported by recent studies in Kenyan children showing that plasma lactate concentrations are associated with the parasite density, IL-12 and Hz-containing neutrophils, a marker of parasite sequestration (Casals-Pascual et al. 2006).

7.6 Electrolyte and Fluid Imbalances

Alterations in electrolyte metabolism are an important biomarker of malaria-associated disturbances in mineral homeostasis. Previous studies showed reduced calcium (calcium <2.13 mmol/L) and phosphate (<1 mmol/L) levels in 23.1% and 38.5% of Nigerian children with malaria, respectively (Ayoola et al. 2005), indicating that hypocalcemia and hypophosphatemia are malaria-associated disturbances. In addition, studies in children admitted to the hospital with severe malaria complicated by acidosis and mild-to-moderate hypercalcemia was common in those with SMA, with severe hyperkalemia associated with complicated malaria and enhanced mortality (Maitland et al. 2005).

A reduced blood volume plays an important role in the severity of malaria and the clinical outcomes of the disease. Hypovolemia is associated with metabolic acidosis and electrolyte imbalances in children with severe malaria (English et al. 1997). In addition, intracellular fluid depletion is associated with increased mortality in Kenyan children with severe malaria (Maitland et al. 2003). Measurements of body compartment volumes in Gabonese children with malaria demonstrated that it is only the total body water volume that has a relationship with enhanced severity of malaria (Planche et al. 2005), suggesting that perturbations in tissue perfusion contributes to the pathogenesis of severe malaria.

7.7 Renal Dysfunction

P. falciparum malaria infections can induce renal dysfunction which manifest as a nephritic syndrome and acute renal failure in both children and adults (WHO 2000). Although renal dysfunction has been observed in African children with uncomplicated malaria (Burchard et al. 2003), renal involvement in malaria is typically associated with severe disease, shock, and electrolyte and hemodynamic disturbances (Maitland et al. 2003; English et al. 1996). One study has shown a relationship between acute renal failure, jaundice, and hepatomegaly in children with malaria (Nacher et al. 2001). Renal dysfunction is also associated with increased mortality in children with CM (Enwere et al. 1999). The mortality associated with severe renal failure commonly occurs in younger children, and those with elevated creatinine levels and reduced urine output (Sheiban 1999). Renal impairment appears to be related to both systemic and inflammatory derangements, as supported by an investigation showing an association between renal dysfunction and circulating levels of TNF-α in children with malaria (Gandapur and Malik 1996).

7.8 Metabolic Acidosis

Metabolic acidosis is an important biochemical derangement that is associated with severe malaria and enhanced mortality. Previous studies showed that 22% of Nigerian

children with CM presented with metabolic acidosis and that this complicating presentation was associated with poor clinical outcomes (Oguche et al. 2002). Consistent with this finding, another investigation showed that metabolic acidosis was prevalent in 21% of Kenyan children with malaria and other acute infections (Sasi et al. 2006). In addition, studies in Kenyan children illustrated that metabolic acidosis was associated with RD and death (English et al. 1996) suggesting that metabolic acidosis is an important complicating factor in severe, life-threatening malaria.

The mechanisms underlying metabolic acidosis remain largely unknown. Although metabolic acidosis has been associated with elevated plasma lactic acid and 3-hydroxybutyric acids (English et al. 1997; Sasi et al. 2007), this clinical chemistry is not synonymous with lactic acidosis since lactate and protons (H^+) are not routinely co-produced (Clark et al. 1997). Protons are formed upon adenosine triphosphate (ATP) hydrolysis (Hotchkiss and Karl 1992). Although aerobic ATP hydrolysis generates protons that are consumed within the mitochondria, anaerobic hydrolysis of ATP leads to proton accumulation and a reduction in pH (Hotchkiss and Karl 1992), suggesting that lactate formation and metabolic acidosis are independent processes. Similar studies also illustrate that plasma pH is not a proxy for lactate in infants with sepsis (Deshpande and Platt 1997). Recent studies indicate high anion and ion gaps in children with malaria with only 40% of the variability in base excess attributable to lactate, creatinine, and inorganic phosphorus (Sasi et al. 2006), suggesting that unidentified ions may be important in the pathogenesis of metabolic acidosis during malaria.

7.9 Respiratory Distress

RD in the context of acute malaria is characterized by alveolar damage and cardiopulmonary alterations, such as pulmonary edema that results from increased alveolar capillary permeability that leads to intravascular fluid loss into the lungs (Taylor et al. 2006b). RD is an important cause of acute lung injury in individuals with severe malaria (Taylor et al. 2006b) and is particularly more common in children presenting with acute malaria in which it can present as a single feature of severe disease or a complicating sequela in the context of SMA and/or CM (Marsh et al. 1995). The prevalence of RD in African children ranges from 23% to 31% during severe, life-threatening malaria (Marsh et al. 1995; Dzeing-Ella et al. 2005). RD is typically indicative of an underlying metabolic acidosis, and is an important predictor of enhanced rates of mortality in children with SMA and/or CM (Marsh et al. 1995). However, the pathophysiological basis for the metabolic abnormalities that cause RD is currently unknown. Although not entirely clear, additional factors that appear to promote RD in children with malaria may be related to co-incidental bacterial sepsis that goes clinically unrecognized (Berkley et al. 1999). Based on this observation, the use of broad spectrum antibiotics in patients presenting with RD appears justified.

While the precise role of immune activation and inflammation in promoting RD during severe malaria is poorly understood, recent studies demonstrate that elevated plasma levels of TNF-α, IL-10, neopterin, and a higher TNF-α/IL-10 ratio are present in

children with RD (Awandare et al. 2006a), suggesting that innate immune activation and inflammatory cytokine dysregulation may be an important etiology of RD.

8 Socioeconomic and Demographic Factors Affecting Severe Malaria

Development of severe malaria, particularly in children, is affected by socioeconomic factors, including nutritional status, family income, caretaker's education level, and birth interval, as well as the intensifying problems in rural sub-Saharan Africa related to affordability and accessibility of preventive and curative health measures (Biemba et al. 2000). Over the past 9 years, our group has been investigating the pathogenesis of SMA in a holoendemic *P. falciparum* transmission region of western Kenya, namely Siaya District, a rural area in Nyanza Province. Although historical measures of the entomologic inoculation rate (EIR) were on the order of 100–300 (Beier et al. 1994), current EIR data are unavailable. However, a recent study demonstrated that pediatric malaria hospital admissions have increased from mid-2007 onward (Okiro et al. 2010) at the primary health facility in the region, Siaya District Hospital (SDH), where our activities are centralized. As with most holoendemic *P. falciparum* transmission areas, SMA is the primary severe clinical manifestation of malaria at SDH, with CM being a rare severe disease manifestation in the population (Ong'echa et al. 2006). Several years ago, we examined the association of clinical, nutritional, demographic, and socioeconomic factors with parasitemia, anemia, and malarial anemia for children presenting at SDH. These investigations revealed that peripheral malaria parasitemia was not associated with malaria disease severity. However, binomial logistic regression revealed that fever was significantly associated with parasitemia, while wasting was associated with enhanced presentation of malarial anemia. Bivariate analyses also showed that caretaker's level of education and occupation were significantly correlated with parasitemia, anemia, and malarial anemia, whereas housing structure was significantly associated with parasitemia and anemia. Interestingly, bed net usage was protective against parasitemia, but not either anemia or malarial anemia. Multivariate logistic regression models demonstrated that fever, mother's occupation, and bed net use were associated with parasitemia, but that none of the factors examined in the comprehensive study were associated with anemia or malarial anemia (Ong'echa et al. 2006). Since this study utilized data collected in 2003–2004 on 374 children, it will be important to determine if these findings are confirmed now that we have more than 1,400 children enrolled in the ongoing investigations.

In addition to infants and young children, other groups in endemic regions of *P. falciparum* transmission are also vulnerable to SMA, such as pregnant women, school-age children, adolescents, and even adults and the elderly, particularly when there is inadequate food intake, nutritional deficiencies, frequent parasitic infestations, and/or complicating co-infections with HIV-1 (Murray et al. 1996; Brabin et al. 2001b; Bronzan et al. 2007; Otieno et al. 2006).

9 Etiological Factors and Clinical Predictors of Severe Malaria

The etiology of SMA can include a number of distinct as well as overlapping features, including lysis of infected and uninfected RBCs (Dondorp et al. 1999a,b; Price et al. 2001; Egan et al. 2002), splenic sequestration of RBCs (Buffet et al. 2009), dyserythropoiesis and bone marrow suppression (Phillips et al. 1986; Abdalla et al. 1980), and co-infections with bacteremia, HIV-1, and hookworm (Otieno et al. 2006; Berkley et al. 2005; Bassat et al. 2009; Davenport et al. 2010; Were et al. 2011), and the chronic transmission of malaria in a holoendemic regions. It is important to note that some or all of these factors can culminate in the chronically low Hb values observed in infants and young children residing in holoendemic regions. As such, the degree of parasitemia is typically a poor indicator of malaria disease severity in these locales, especially considering that peripheral parasitemia is a "snapshot" in time of the complex and continuously evolving disease process. However, it is important to stress that high levels of parasitemia, particularly in nonimmune individuals can certainly lead to massive lysis and clearance of RBCs, resulting in profound anemia (Phillips et al. 1986; Molyneux et al. 1989).

Several comprehensive studies have recently been conducted to explore the potential factors associated with pediatric malarial anemia in sub-Saharan Africa. One of these investigations utilized a case–control design in 381 Malawian preschool children with severe anemia (Hb < 5.0 g/dL) and 757 preschool children without severe anemia residing in both urban and rural settings (Calis et al. 2008). This investigation revealed that bacteremia, malaria, hookworm, HIV, G6PD (−202/−376), vitamin A deficiency, and vitamin B12 deficiency were all associated with an increased risk of severe anemia (Calis et al. 2008). Interestingly, malaria was associated with severe anemia in the urban site with seasonal transmission, but not in the rural site that had holoendemic *P. falciparum* transmission (Calis et al. 2008).

The second study, recently conducted by our group in a holoendemic region of western Kenya, took the perspective that an important strategy for reducing the morbidity and mortality associated with SMA is to identify clinical predictors that can be readily recognized by caregivers for prompt therapeutic interventions. As such, we determined the clinical predictors of SMA in Kenyan children (3–36 months, $n = 671$) presenting with acute illness at SDH (Novelli et al. 2010). For this study, demographic, clinical, laboratory, and hematological parameters were measured upon presentation at hospital. Because we have shown that HIV-1 and bacteremia can potentially augment anemia in this region (Otieno et al. 2006; Davenport et al. 2010; Were et al. 2011), all study participants were screened for those diseases and excluded from the analyses. Children with *P. falciparum* ($n = 355$) were stratified into three groups: uncomplicated malaria (Hb ≥ 11.0 g/dL); non-SMA (6.0 ≤ Hb < 10.9 g/dL), and SMA (Hb < 6.0 g/dL). This study revealed that SMA was characterized by a younger age, monocytosis, thrombocytopenia, reticulocytosis, reduced erythropoiesis, elevated pigment-containing monocytes (PCM), RD, conjunctival and palmar pallor, splenomegaly, signs of malnutrition, and protracted fever and emesis. Modeling with logistic regression analysis demonstrated that age,

reticulocyte count, presence of PCM, and conjunctival and palmar pallor were significant predictors of SMA. This study confirmed one of the primary themes that has emerged from our studies in this region over the past decade i.e., children with SMA have lower peripheral parasite densities than parasitemic children without anemia (Hb ≥ 11.0 g/dL) (Ong'echa et al. 2006; McElroy et al. 2000), suggesting that acute hemolysis of RBCs is not likely the primary cause of low Hb levels observed in children with SMA in this holoendemic transmission region.

10 Role of Innate Immunity in Malaria Pathogenesis

The host releases an array of pro- and anti-inflammatory cytokines, chemokines, growth factors, and effector molecules as part of the innate immune response to malaria. The clinical outcomes in response to a malaria infection are, therefore, largely influenced by the relative balance of inflammatory mediators released by the human host. Depending on the magnitude and timing of inflammatory mediator release, the immune response to malaria can either successfully control the parasitemia or, alternatively, generate an inflammatory milieu that can cause damage to the host. However, it is important to note that clarification of strict "protective" versus "pathological" roles for inflammatory mediators remain poorly defined and extremely difficult to quantify in human malaria in which manipulation of the biological systems is typically not practical. There are a number of key parasitic products that drive the innate immune response to malaria such as malarial pigment (hemozoin, Hz), glycosylphosphatidylinositols (GPIs), and parasitic antigens.

10.1 Pro-inflammatory Mediators

A successful type 1 response to malaria requires a well-timed and proportional release of IL-12, interferon (IFN)-γ, and TNF-α to minimize parasitemia (Crutcher et al. 1995; Stevenson et al. 1995). For proper immunological control, the pro-inflammatory phase should be followed by an equally timely abrogation of the type 1 response via type 2 cytokines such as IL-10, transforming growth factor (TGF)-β, and IL-4 to avoid inflammation-mediated damage to the host (Clark et al. 2006).

TNF-α is the prototypical molecule associated with enhanced pathology during a malaria infection, and was first hypothesized to be an important component of the host immune response to malaria in 1978 (Clark 1978). Although elevated TNF-α levels are often associated with adverse clinical outcomes in individuals with malaria (Grau et al. 1989; Kwiatkowski et al. 1990), TNF-α is critical for controlling parasitemia (Grau et al. 1989; Kwiatkowski et al. 1989, 1990; Kern et al. 1989; Clark et al. 1990). TNF-α also mediates its effects by inducing production of macrophage migration inhibitory factor (MIF) (Calandra and Bucala 1995; Lan et al. 1997) and nitric oxide synthase type 2 (NOS2, inducible nitric oxide synthase,

iNOS) (Rockett et al. 1992). Induction of NOS2 generates high levels of nitric oxide (NO) production that has direct parasite killing effects (Rockett et al. 1991). Many of the signs and symptoms associated with malaria such as fever, headache, nausea, vomiting, diarrhea, anorexia, myalgias, and thrombocytopenia are associated with enhanced TNF-α production (Schwartz et al. 1989).

IFN-γ is produced by natural killer cells, αβ-T cells, and regulatory γδ-T cells during the initial phase of the immune response to malaria (Hensmann and Kwiatkowski 2001; Artavanis-Tsakonas and Riley 2002; D'Ombrain et al. 2007). Release of IFN-γ is important for protection against malaria during natural infections (D'Ombrain et al. 2008) and in nonimmune volunteers experimentally infected with malaria (Pombo et al. 2002). Consistent with a protective role, IFN-γ responses to CD8+ T cell epitopes from pre-erythrocytic antigens are associated with higher Hb levels, and reduced prevalence of severe malaria (Ong'echa et al. 2003).

Together, TNF-α and IFN-γ play protective roles during the early stages of a *P. falciparum* infection through their ability to stimulate monocyte/macrophage activation and control parasitemia (Kremsner et al. 1995). However, overproduction of these inflammatory mediators can promote adverse clinical outcomes such as malaria-associated anemia (Lyke et al. 2004; Perkins et al. 2000) as evidenced by the fact that persistent macrophage activation is associated with more complicated forms of clinical malaria (Biemba et al. 1998). Sustained overproduction of IFN-γ, TNF-α, and NO can also lead to malarial anemia through their ability to cause bone marrow suppression, dyserythropoiesis, and erythrophagocytosis (Clark and Cowden 2003).

IL-1 is an endogenous pyrogen released as part of innate immunity that provides defense against an array of pathogens (Dinarello 2004). In murine models of malaria, IL-1β and IL-1α synergize with TNF-α to enhance the production of IFN-γ and NO (Rockett et al. 1994). High levels of sustained IL-1β production, however, can induce hematological abnormalities such as anemia (Pascual et al. 2005; Dinarello 2005). Administration of recombinant IL-1 in murine models of malaria inhibits the development of pre-erythrocytic stages of malaria (Pied et al. 1992), protects against CM, and helps to limit parasitemia (Curfs et al. 1990). The published data on IL-1β in human malaria has yielded mixed results with several reports showing elevated circulating IL-1β levels in cases of severe malaria (Prakash et al. 2006; Vogetseder et al. 2004; John et al. 2006), and another illustrating no significant differences in IL-1β levels in children with severe disease (Lyke et al. 2004). However, comprehensive studies by our group in a large group of Kenyan children showed that SMA was associated with significantly lower circulating levels of IL-1β compared to malaria-parasitized children without SMA (Ouma et al. 2008a). Haplotypic construction of IL-1β promoter polymorphisms revealed that carriage of haplotypes that conditioned increased risk to developing SMA was also associated with decreased IL-1β production (Ouma et al. 2008a). Taken together, it appears that although previous data suggests that sustained IL-1β production has the capacity to promote anemia (Pascual et al. 2005; Dinarello 2005), high producing IL-1β promoter haplotypes also can protect against severe anemia through a yet, undiscovered mechanism(s).

More than two decades ago, it was recognized that peripheral blood levels of IL-6 are elevated in patients with severe *P. falciparum* malaria (Kern et al. 1989) with subsequent studies supporting this initial finding (Kremsner et al. 1995; Lyke et al. 2004; John et al. 2006). During an acute malaria episode, peripheral blood mononuclear cells (PBMCs) are a primary source of increased IL-6 production (Aubouy et al. 2002). However, murine models of malaria demonstrate beneficial effects of IL-6 with IL-6 providing protective immunity against the pre-erythrocytic stages of malaria through its ability to augment IL-1β and TNF-α production (Pied et al. 1992). IL-6 in these model systems also provides protective effects during the erythrocytic stage of disease by boosting specific immunoglobulin (Ig) G antibodies that help control parasitemia. Protective effects of IL-6 have also been shown in humans experimentally infected with *P. falciparum* (Harpaz et al. 1992). Consistent with the protective effects of IL-6, reduced blood levels of IL-6 are associated with hyperparasitemia in children with falciparum malaria (Lyke et al. 2004). Taken together, previous studies support a protective role for IL-6 during the early stages of malaria infections by controlling parasitemia. In addition, prior investigations also support a model in which lack of control over parasitemia during the early stages of an infection, and the subsequent progression towards severe disease, continues to drive high levels of sustained IL-6 that can promote enhanced pathophysiology.

MIF was the first soluble mediator described in malaria (Coleman et al. 1976). After several decades of only a limited number of investigations into the role of MIF in malaria pathogenesis, MIF has been revisited as a potentially important inflammatory mediator in malaria by our group and a number of others (Awandare et al. 2006b,c, 2007, 2009; De Mast et al. 2008; Jain et al. 2009). MIF is a ubiquitous cytokine produced in response to pro-inflammatory stimuli by T cells (David 1966; Bacher et al. 1996), monocytes/macrophages (Calandra and Roger 2003), and the anterior pituitary gland (Bernhagen et al. 1998). However, unlike most cytokines, MIF is constitutively expressed at high levels and stored in preformed vesicles, and as such, can be rapidly released without de novo gene expression (Bernhagen et al. 1993, 1998). MIF has potent pro-inflammatory properties that govern both innate and adaptive immune responses to bacterial and parasitic infections (Bacher et al. 1996; Calandra and Roger 2003; Calandra et al. 2000; Koebernick et al. 2002; Juttner et al. 1998). Murine models of malaria show that elevated MIF levels are associated with more profound disease severity (Martiney et al. 2000). This notion is supported by the fact that MIF gene knockout mice have less severe anemia and enhanced survival following infection with *Plasmodium chabaudi* relative to wild-type mice (McDevitt et al. 2006).

Previous studies show that MIF is elevated in humans with placental malaria (Chaisavaneeyakorn et al. 2003; Chaiyaroj et al. 2004), in the thoracic blood vessels of children with CM (Clark et al. 2003) and in the peripheral blood of children with acute malaria (McDevitt et al. 2006). In contrast, results from our laboratory were the first to demonstrate that elevated MIF protein (in circulation) and MIF transcripts (in PBMCs) were associated with less severe forms of falciparum malaria in Gabonese children (Awandare et al. 2006b). Our subsequent results in a larger cohort of Kenyan children (aged <3 years, $n = 357$) showed that MIF is indeed

suppressed during *P. falciparum* infections with low levels of MIF associated with more severe forms of malarial anemia (Awandare et al. 2007). For example, circulating MIF concentrations declined with increasing severity of anemia and significantly correlated with peripheral blood leukocyte MIF transcripts (Awandare et al. 2007). Additional experiments conducted in cultured PBMC from malaria-naïve individuals showed that phagocytosis of *P. falciparum* malarial pigment (hemozoin, *Pf*Hz) was the source of altered MIF production through an apoptosis-independent mechanism (Awandare et al. 2007). Thus, elevated levels of *Pf*Hz acquired during a malarial infection promote suppression of peripheral blood MIF levels that correlate with enhanced severity of anemia.

IL-23 is another pro-inflammatory mediator that appears important in conditioning the pathogenesis of severe malaria. Although largely unexplored in the context of malaria, IL-23 is important in mediating the development of anemia in autoimmune diseases (Cua et al. 2003) and chronic inflammation (Wiekowski et al. 2001). IL-23 is composed of two subunits, p19 and p40 (Oppmann et al. 2000). IL-23 shares a number of common properties with IL-12, including the p40 subunit (Shimozato et al. 2006), the ability to bind to the IL-12Rβ1 receptor (Parham et al. 2002), release from activated myeloid antigen presenting cells, promotion of a type 1 immune response (Oppmann et al. 2000; Shimozato et al. 2006; Parham et al. 2002; Pirhonen et al. 2002; Trinchieri 2003), and suppression by both IL-10 (Aste-Amezaga et al. 1998; Schuetze et al. 2005) and IL-12p40 homodimers (Shimozato et al. 2006; Gately et al. 1996). In addition to the common properties IL-23 shares with IL-12, there are also distinct immunological roles in that IL-23 acts on activated memory CD4+ T cells, while IL-12 promotes Th1 differentiation of naïve CD4+ T cells (Oppmann et al. 2000; Aggarwal et al. 2003). Based on the common and distinct roles of IL-23 and IL-12, along with the well-established importance of IL-12 in the pathogenesis of malarial anemia (discussed below in detail), we explored the relationships among these cytokines in Kenyan children with varying severities of malarial anemia (Ong'echa et al. 2008). Children with malarial anemia had increased peripheral blood levels of IL-23 and suppressed levels of IL-12 relative to healthy controls. Experiments in cultured PBMC revealed that *Pf*Hz caused a sustained induction of IL-23p19 transcripts over 72 h, while IL-12p40 and IL-10 transcripts peaked at 24 h, and rapidly declined thereafter. Thus, it appears that elevated levels of IL-23 may play an important role in the pathogenesis of SMA, and that both IL-10 and IL-12 appear to regulate IL-23 production during an infection with *P. falciparum*.

IL-12, a heterodimeric protein composed of 35 and 40 kDa subunits and a prototypical cytokine of the type 1 immune response (Gately et al. 1998; Trinchieri 1998), is perhaps the most critical innate inflammatory mediator for mediating malaria pathogenesis. IL-12 is secreted from dendritic cells, monocytes, and B-cells in response to bacterial cell wall components, intracellular pathogens, and CD40 ligation (Gately et al. 1998; Trinchieri 1998; Mosser and Karp 1999). IL-12 stimulates production of IFN-γ and TNF-α from T-cells and natural killer (NK) cells (Gately et al. 1998; Trinchieri 1998), thereby, further augmenting type 1 responses. A number of inflammatory mediators promote IL-12 [e.g., granulocyte macrophage-colony

stimulating factor (GM-CSF) and IFN-γ], while others suppress IL-12 [e.g., IL-4, IL-10, IL-11, IL-13, monocyte chemotactic protein (MCP)-1/CCL2, and TGF-β] (Trinchieri 1998; Mosser and Karp 1999). Administration of recombinant IL-12 and chloroquine ameliorates blood-stage disease and severe anemia, and provides protection against reinfection in murine models of malaria (Mohan et al. 1999). In such models, reduced IL-12 production is also associated with enhanced severity of anemia and dyserythropoiesis (Mohan and Stevenson 1998). The protective responses associated with IL-12 against blood-stage malaria are related to elevated production of IL-12 from splenic macrophages and NK cells (Mohan et al. 1997; Sam and Stevenson 1999) and the ability of IL-12 to stimulate antibody production (Su and Stevenson 2002).

Central to the role of IL-12 in malaria is its ability to act as a hematopoietic growth factor (Bellone and Trinchieri 1994; Dybedal et al. 1995). During times of cytopenic crisis, IL-3, IL-6, IL-11, IL-12, and GM-CSF promote colony formation of dormant hematopoietic progenitors (Bellone and Trinchieri 1994; Dybedal et al. 1995). Consistent with this role, previous investigations from our group demonstrated that severe anemia in children with falciparum malaria is characterized by suppressed levels of IL-12 (Perkins et al. 2000; Luty et al. 2000) due to increased phagocytosis of *Pf*Hz by monocytes (Luty et al. 2000). Reduced IL-12 levels during malaria occurs through a mechanism that involves phagocytosis of *Pf*Hz, an event that promotes increased levels of monocyte-derived IL-10 that, in turn, suppress IL-12p40 subunits (Keller et al. 2006a).

10.2 Anti-inflammatory Mediators

Elevated levels of anti-inflammatory cytokines, such as IL-10, prevent the overproduction of pro-inflammatory mediators and down-regulate the potentially pathogenic type 1 (pro-inflammatory) responses during a malaria infection (Ho et al. 1998). For example, a high IL-10 to TNF-α ratio is associated with less severe forms of childhood malarial anemia (Perkins et al. 2000; Othoro et al. 1999). It is, therefore, the timing and magnitude of pro-inflammatory cytokine production, relative to the anti-inflammatory cytokine response, that conditions, at least in part, the clinical outcomes of malaria. Studies in Malian children with SMA show that IL-10 levels are elevated relative to healthy controls (Lyke et al. 2004), while other investigations in Ghanaian children illustrate that plasma IL-10 levels are lower in cases of SMA compared to children with CM, uncomplicated malaria, or moderate malarial anemia (Kurtzhals et al. 1998). Based on the significant positive relationship between plasma IL-10 concentrations and pigment-containing leukocytes in circulation (Luty et al. 2000), it appears that phagocytosis of malarial pigment plays an important role in determining IL-10 production during a falciparum infection.

TGF-β1 is an anti-inflammatory cytokine (and growth factor), which down-regulates the production of TNF-α and IL-10, and protects against severe disease in murine models of malaria (Omer and Riley 1998). TGF-β appears to be important

in human malaria pathogenesis (Prakash et al. 2006; Chaiyaroj et al. 2004; Gourley et al. 2002), and is associated with both positive (Zermati et al. 2000) and negative effects (Zermati et al. 2000; Hino et al. 1988; Sing et al. 1988) on erythropoietic cascade. We have previously shown that circulating TGF-β1 levels are significantly reduced in children with severe malaria (Perkins et al. 2000). However, other investigations have shown opposite effects in which severe childhood malaria is characterized by increased circulating levels of TGF-β1 (Malaguarnera et al. 2002). The reason for differing results may be related to differences in malaria endemicity since our study was conducted in a rural site in Lambaréné, Gabon, with a high level of *P. falciparum* transmission, whereas the results in Ouagadougou, Burkina Faso, were from an urban region with mesoendemic *P. falciparum* transmission. Furthermore, it is unclear whether "platelet poor" samples were generated in the study in Burkina Faso: "platelet poor" samples are recommended because platelets contain high levels of TGF-β1 that can generate inaccurate peripheral blood measurements of TGF-β1.

In addition to TGF-β1, a recent investigation in Angolan children illustrates that polymorphic variability in TGF-β2 conditions susceptibility to the risk of progressing to CM (Sambo et al. 2010). A recent study also supports the importance of the TGF-β family in malaria pathogenesis by showing that serum levels of the soluble form of the TGF-β co-receptor, endoglin (sEng or CD105/TGF-βRIII), was significantly elevated in children with severe falciparum malaria (Dietmann et al. 2009).

10.3 Chemokines

Chemotactic cytokines, or chemokines, are primarily known for their chemotactic properties, but also play important roles in immune activation, hematopoiesis, angiogenesis, and antimicrobial activities (Rollins 1997). IL-8/CXCL8 is an important neutrophil activating chemokine that is elevated in Thai patients with severe, non-fatal malaria (Friedland et al. 1993). The first study to investigate chemokines in human malaria also showed that circulating IL-8/CXCL8 concentrations are positively correlated with parasitemia levels in adults with *P. falciparum* malaria (Burgmann et al. 1995). A subsequent investigation revealed that children with severe malaria had tenfold higher concentrations of IL-8/CXCL8 compared to either healthy controls or individuals with uncomplicated malaria (Lyke et al. 2004). Additional studies in Gabonese children and adults illustrate that high levels of IL-8/CXCL8 are associated with acute infections and a slow rate of cure following malaria chemotherapy (Kremsner et al. 1995).

Additional studies on chemokines from our group demonstrated that Gabonese children with severe malaria had elevated levels of macrophage inflammatory protein (MIP)-1α/CCL3 and MIP-1β/CCL4 protein (measured in circulation) and transcripts (determined in ex vivo PBMC) (Ochiel et al. 2005). It appears that phagocytosis of *Pf*Hz is an important signal for promoting chemokine production

and/or suppression during a malaria infection. We have previously shown that treatment of cultured PBMC from healthy, malaria-naïve donors with *Pf*Hz increases MIP-1α/CCL3 and MIP-1β/CCL4 production (Ochiel et al. 2005). These results are consistent with a study demonstrating that *Pf*Hz treatment of a bone marrow-derived murine cell line causes elevated MIP-1α/CCL3, MIP-1β/CCL4, MIP-2/CXCL2, and monocyte chemotactic protein (MCP)-1/CCL2 transcript levels (Jaramillo et al. 2005).

Regulated on activation, normal T-cell expressed and secreted (RANTES/CCL5) also plays an important role in malaria pathogenesis. RANTES is secreted by a number of cell types including monocytes, macrophages, fibroblasts, NK and T cells, and CD34+ hematopoietic progenitors (Conlon et al. 1995; Marfaing-Koka et al. 1996; Mariani et al. 2002; Umland et al. 2004). RANTES protein is sequestered in the α-granules of platelets and released upon thrombin stimulation (Tang et al. 2002). RANTES stimulates hematopoiesis, angiogenesis, cell proliferation, and development (Luster 2002). An earlier study by our group, which was the first to examine RANTES in the context of malaria, demonstrated that RANTES was suppressed in Gabonese children with severe malaria, at least in part, through *Pf*Hz-induced down-regulation of RANTES transcripts in PBMC (Ochiel et al. 2005). The inherent ability to produce RANTES/CCL5 also appears important in conditioning susceptibility to severe malaria. For example, our investigation in Gabon revealed that healthy children with prior mild malaria produced significantly higher RANTES transcripts and protein than children with a history of severe malaria (Ochiel et al. 2005). These investigations were then confirmed in Kenya where we showed that RANTES was significantly suppressed in children with SMA (Were et al. 2006). Suppression of RANTES in these children was associated with inefficient erythropoiesis and malaria-induced thrombocytopenia (Were et al. 2006). Subsequent studies from our laboratories determined that naturally acquired *Pf*Hz by monocytes promotes suppression of RANTES in children with malarial anemia through an IL-10-dependent mechanism (Were et al. 2009). Taken together, these findings suggest that thrombocytopenia may be an important source of reduced RANTES in children with malaria which contributes to inefficient erythropoiesis in children with severe disease.

10.4 Growth Factors

The literature is largely deficient with respect to the importance of growth factors in conditioning the development of severe malaria. One time-course study in patients with *P. falciparum* malaria in Thailand showed that serum levels of granulocyte-colony stimulating factor (G-CSF) were significantly elevated in individuals with complicated disease on day 0, and subsequently declined to within the normal range by day 7 (Stoiser et al. 2000). Levels of G-CSF on day 0 were correlated with procalcitonin, parasite density, and erythropoietin (Stoiser et al. 2000). Although to date, G-CSF has not been examined in children with SMA, such studies are warranted since over-production of G-CSF can decrease erythropoiesis (Van Zant and Goldwasser 1977; Kojima et al. 1991; Papaldo et al. 2006).

GM-CSF synergizes with TNF-α to increase the killing capabilities of neutrophils for the elimination of blood-stage malaria parasites (Kumaratilake et al. 1996), and is important for promoting erythropoiesis (Liehl et al. 1994). In a murine model of malaria, enhanced pathology, characterized by elevated parasitemia and anemia, is associated with elevated erythropoietin (EPO), a strong correlate of anemia severity, and negatively correlated with GM-CSF concentrations (Chang and Stevenson 2004). The impact of dysregulation in GM-CSF on the clinical outcomes in human malaria remains largely undetermined.

Our previous investigations employed a strategy of (macroarray) gene expression profiling of pooled fractions of human PBMCs stimulated with *Pf*Hz to identify genes/gene pathways that play a role in the pathogenesis of malaria. These experiments revealed that human stem cell growth factor [SCGF, C-type lectin domain family member 11A (CLEC11A)] was up-regulated following treatment with *Pf*Hz (Keller et al. 2009). SCGF is a hematopoietic growth factor, expressed primarily by myeloid cells and fibroblasts that possess burst-promoting activity for human bone marrow erythroid progenitors (Hiraoka et al. 2001). Human SCGF-α is a 323-amino acid protein, while SCGF-β is a 245-amino acid protein that results from cleavage of the conserved carbohydrate domain (Mio et al. 1998). After determining the in vitro kinetics of SCGF expression in response to *Pf*Hz, we then examined circulating SCGF levels in Kenyan children with malarial anemia. SCGF levels in circulation and in cultured peripheral blood were significantly suppressed in children with SMA, with circulating SCGF levels being positively correlated with Hb concentration and the reticulocyte production index (Keller et al. 2009). SCGF was significantly lower in children with a suppressed erythropoietic response and in children with high levels of naturally acquired monocytic *Pf*Hz (Keller et al. 2009). An additional investigation in the same cohort of children with *P. falciparum* malaria showed that a novel SCGF promoter variant (−539 C/T, rs7246355) was significantly associated with susceptibility to SMA and reduced erythropoietic responses with the "high producing" TT genotype protecting against development of SMA and suppression of erythropoiesis (Ouma et al. 2010). Thus, SCGF is an important mediator of SMA pathogenesis that could offer the potential for immunotherapy in future clinical trials.

10.5 Effector Molecules

As described above, the clinical outcomes of malaria are largely conditioned by the relative expression of inflammatory mediators. The relative timing and magnitude of pro- and anti-inflammatory cytokines, chemokines, and growth factors released into the inflammatory milieu have direct actions on the cellular response as well as the "down-stream" effector molecules that ultimately get produced. As such, effector molecules play a critical role in the pathogenesis of malaria. One important effector molecule in malaria is the toxic free radical, NO. NO and equimolar amounts of L-citrulline are generated by catalysis of L-arginine by the NO synthases (NOS)

(Nathan and Xie 1994). In an acute inflammatory disease, such as malaria, high levels of NO are derived from the cytokine inducible isoform, nitric oxide synthase type 2 [NOS2 or inducible NO synthase (iNOS)] present in monocytes, macrophages, and neutrophils (Perkins et al. 1999). Pro-inflammatory cytokines such as IL-12, IFN-γ, and TNF-α increase NOS2-generated NO production, while anti-inflammatory cytokines such as IL-10 and TGF-β down-regulate NOS2 expression (for review see Geller and Billiar 1998). Although the role of NO in the pathogenesis of malaria has been debated for over a decade, it is clear that high levels of NOS2-generated NO has both protective and pathogenic properties. For example, NO is protective because it has potent parasiticidal properties against *P. falciparum* (Rockett et al. 1991) and can thereby aid in controlling parasitemia (Kremsner et al. 1996). Protective properties are also illustrated by our previous investigation in healthy, malaria-exposed Gabonese children with a history of mild malaria that possess significantly higher levels of ex vivo PBMC NO production and NOS enzymatic activity relative to their age-matched cohort with a history of severe malaria (Perkins et al. 1999). Pathogenic effects are exemplified by our follow-up investigation in the same population of children in which ex vivo and in vitro PBMC NOS activity was significantly higher in children with malarial anemia: there was an inverse association between NOS enzyme activity and hemoglobin levels (Keller et al. 2004a). Additional experiments confirmed that *Pf*Hz was an important source for generating elevated levels of NOS2 transcripts and NO production (Keller et al. 2004a). Thus, although NO serves an important role in controlling parasitemia, it is likely that sustained, high levels of NO production can also promote anemia and tissue damage. This hypothesis is supported by the fact that over-production of NO during a malarial infection can promote severe anemia through bone marrow suppression, dyserythropoiesis, and erythrophagocytosis (for review see Clark and Cowden 2003).

Reactive oxygen species (ROS) are also both protective and pathogenic in human malaria. Protective properties are illustrated by studies showing that high levels of oxygen radical production are associated with accelerated clearance and control of *P. falciparum* parasitemia (Greve et al. 1999; Postma et al. 1996). Conversely, a pathogenic role for ROS is illustrated by a study in Kenyan children showing that ROS cause damage to the erythrocytic membrane (demonstrated by measurement of α-tocopherol and polyunsaturated fatty acid levels in the erythrocyte membrane) (Griffiths et al. 2001). A recent investigation in Indian children (<15 years of age) infected with *P. falciparum* further revealed that severe malaria cases were characterized by significantly elevated markers of oxidant stress, including malondialdehyde, protein carbonyl, nitrite, ascorbic acid, and copper levels (Narsaria et al. 2011). Although a wealth of data exists on the topic of free radicals in malaria, such a discussion is beyond the scope of the current book chapter. However, the selected studies presented here illustrate the fact that reactive nitrogen and oxygen intermediates possess both protective and pathogenic properties.

Prostaglandin (PG)E_2 is synthesized from arachidonic acid (AA) through the catalytic activity of cyclooxygenase (COX) enzymes also known as prostaglandin-H_2 (PGH_2) synthase, which exists in two isozymes: COX-1 (PGH synthase-1) and

COX-2 (PGH synthase-2). Constitutively expressed COX-1 catalyzes immediate biosynthesis of PGE_2 and other prostanoids involved in physiological homeostasis, whereas inducible COX-2 catalyzes delayed formation of PGE_2 and prostanoids involved in regulating the inflammatory response and immunity to invading pathogens (Vane et al. 1998). Formation of PGH_2, the committed step in prostanoid biosynthesis, promotes generation of primary prostanoids [i.e., PGE_2, PGH_2, thromboxane A_2, PGD_2, $PGF_{2\alpha}$, and prostacyclin (PGI_2)] through the action of respective terminal prostanoid synthases (Vane et al. 1998). Our previous study illustrates that intervillous blood mononuclear cell (IVBMC) PGE_2 production is reduced in parasitemic women of all gravidae due, at least in part, to acquisition of intraleukocytic *Pf*Hz (Perkins et al. 2003). Additional studies from our group have shown that plasma bicyclo-PGE_2 (a stable end metabolite of PGE_2) and ex vivo PBMC COX-2 gene expression are significantly reduced in Gabonese children with severe malaria (Perkins et al. 2001). Studies in Tanzanian children also showed that suppression of systemic bicyclo-PGE_2 production (measured in urine) was suppressed in children with CM (Perkins et al. 2005). In addition, in vitro experiments in our laboratories revealed that reduced PGE_2 biosynthesis in children with falciparum malaria was largely due to inhibition of de novo COX-2 transcripts following phagocytosis of *Pf*Hz by monocytes (Keller et al. 2004b, 2006b). Further investigation of the role of prostaglandins in childhood malaria showed that suppression of PGE_2 by *Pf*Hz and commonly used antipyretics to treat the malarial fever promoted over-production of TNF-α, an event associated with enhanced malaria pathogenesis (Keller et al. 2004b, 2006b).

10.6 Model of Inflammatory Mediator-Mediated Pathogenesis in SMA

As an illustrative example of how altered levels of inflammatory mediators can promote the pathogenesis of SMA, we have constructed a model based on our investigations examining dysregulation in pro- and anti-inflammatory cytokines, chemokines, growth factors, and effector molecules in children with falciparum malaria in western Kenya (Fig. 8.3).

10.7 Proposed Model of Dysregulation in Innate Immune Responses in SMA

Based on concomitant measurement of innate inflammatory mediators (using multiplex technologies) in children with varying severities of malarial anemia, we developed a model to describe how dysregulation in innate inflammatory mediators promotes suppression of erythropoiesis in children with SMA. Central to the model is the fact

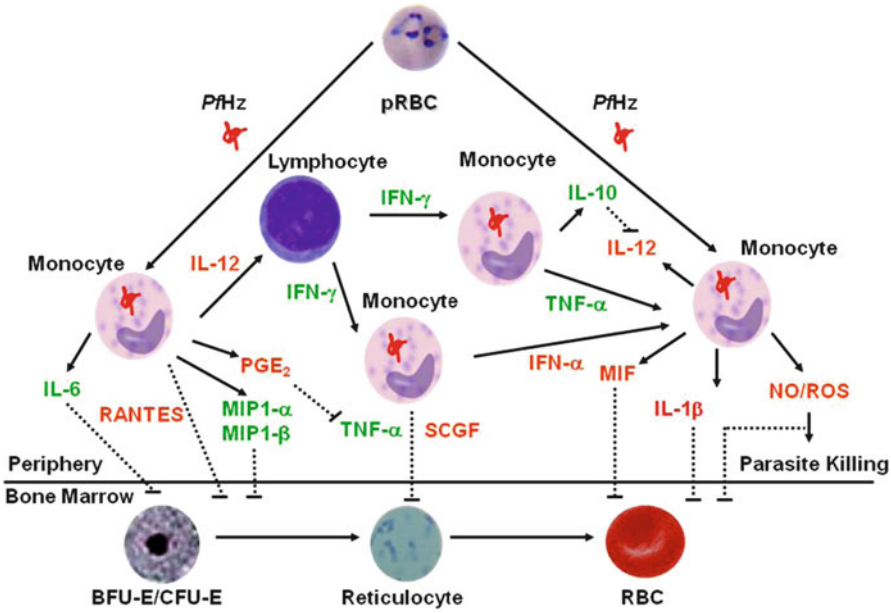

Fig. 8.3 Model of severe malarial anemia pathogenesis

that phagocytosis of hemozoin (*Pf*Hz) by monocytes is one of the primary causes of altered production of innate inflammatory mediators. Elevated inflammatory mediators are shown in green text, while those that are decreased in children with SMA are shown in red text. Solid lines indicate positive signaling (up-regulation), whereas dashed lines indicate suppression (down-regulation). Children with SMA have decreased levels of IL-12 in response to ingestion of *p*RBCs and/or hemozoin by monocytes. Suppression of IL-12 in children with SMA is due to *Pf*Hz-induced IL-10 over-production. Children with SMA have increased circulating levels of TNF-α, IFN-γ, IL-6, MIP-1α, and MIP-1β. Although TNF-α can induce PGE_2 and nitric oxide (NO), these effector molecules are suppressed in children with SMA. Suppression of PGE_2 allows over-production of TNF-α, which is associated with enhanced severity of anemia. In addition, MIF is suppressed in children with falciparum malaria, an event associated with phagocytosis of *Pf*Hz by monocytes, and enhanced severity of anemia. Circulating levels of IFN-α, IL-1β, RANTES, and SCGF are also decreased in children with SMA. Reduced production of these innate inflammatory mediators, along with increased TNF-α, IL-6, MIP-1α and MIP-β, likely contribute to the development of SMA by suppressing the erythropoietic response. Lastly, although the reduced NO and ROS generation reported in children with falciparum malaria may promote ineffective parasite killing and, thereby, prolong parasitemia, children with malarial anemia have elevated levels of NO and ROS that can directly inhibit erythropoiesis.

11 Acquired Immunity to Malaria

Individuals residing in endemic areas acquire immunity to *P. falciparum* malaria following repeated infections that, in most cases, results in control of parasite density and a reduction in the frequency of clinical malaria episodes (Hviid 2005). Regulation of parasite prevalence (and density) through acquisition of immunity is governed by both the rate of exposure to the parasite and intrinsic age-related immune factors (Baird 1995). Previous epidemiological studies support the premise that acquired immunity to malaria develops rapidly after 1 year of age (Maire et al. 2006). However, careful inspection of age-related patterns showing decreasing levels of parasitemia with increasing age provides evidence that acquired immunity progressively develops between infancy and adulthood (Baird 1995).

Both cellular and antibody-mediated immune responses are important for the development of acquired immunity in individuals residing in malaria endemic areas. IgG antibodies play a critical role in protection against the asexual blood stages of *P. falciparum* as demonstrated by improved clinical outcomes in malaria-infected individuals receiving passive transfer of IgGs from immune-competent adults (Sabchareon et al. 1991). Immuno-epidemiological studies show that the cytophilic IgG_1 and IgG_3 subclasses, rather than the non-cytophilic IgG_2 and IgG_4 isotypes, are important for antimalarial immunity (Shi et al. 1999). Protective immunity occurs, at least in part, through monocyte-mediated antibody-dependent cell-mediated inhibition (ADCI) of asexual blood stage merozoites (Shi et al. 1999). The primary mechanism of ADCI is mediated through IgG_1 and IgG_3 binding to monocytes expressing FcγRI and FcγRII receptors which enhances opsonization and phagocytosis (Bouharoun-Tayoun et al. 1995). Studies in semi-immune adults in Kenya and Gabon confirm that IgG_1 and IgG_3 are important elements of the monocyte-mediated ADCI of asexual blood stage *P. falciparum* parasites (Shi et al. 1999).

Based on the fact that ADCI largely utilizes *P. falciparum*-specific IgG_1 and/or IgG_3, structural differences in the FcγRs, particularly FcγRIIa (CD32) allotypes play an important role in susceptibility to severe malarial. For example, studies in Thailand show that the FcγRIIa −131 H/H genotype, in combination with the FcγRIIIb −NA2 allele, condition susceptibility to CM (Omi et al. 2002), and an investigation in Gambian children illustrates that −131 H/H is associated with susceptibility to severe malaria (Cooke et al. 2003). Studies by our group and others also show that carriage of FcγRIIa −131R protects infants and children against high-density *P. falciparum* parasitemia (Ouma et al. 2006; Shi et al. 2001).

The cellular arm of the adaptive immune response to malaria is dependent on various subsets of T lymphocytes and antigen-presenting cells (APCs). An enhanced capacity of CD3+ cells to produce IFN-γ and TNF-α in response to late-stage schizont-rich parasites is an important component of protective immunity (Ramharter et al. 2005). In addition, studies conducted in Cameroonian children illustrate that protective cellular immunity to *P. falciparum* involves an early Th_2-type response (characterized by IL-4 production), and the subsequent switch to a Th_1-type response (characterized by IFN-γ production) (Le Hesran et al. 2006). Studies in children

with acute malaria showing an inverse association between CD4+ T cells and blood-stage parasite densities (Lisse et al. 1994), along with the association between expansion of CD4+ and CD8+ T cells producing IFN-γ and enhanced clearance of blood-stage parasitemia (Winkler et al. 1998), demonstrate the important role of CD4+ and CD8+ T cells in regulating cellular immunity to malaria.

12 Host Genetic Factors Conditioning Susceptibility to Severe Malaria

Recent advances in human gene mapping (e.g., human genome and International HapMap projects), along with an increased understanding of the molecular immune mechanisms of protective immunity, illustrate that susceptibility to malaria (and the accompanying clinical outcomes) is conditioned by genotypic variation. Studies of genetic variation can utilize evolutionarily stable DNA markers to analyze candidate susceptibility genes including single base pair (bp) variations i.e., single-nucleotide polymorphisms (SNPs), occurring at approximately one in every 300–1,000 bp, microsatellite or minisatellites (i.e., variable number tandem repeat; VNTRs), and copy number variations (CNVs). The human genome project revealed the enormous amount of inter-individual variation in nucleotide sequence present in the human population. Among the many types of variation occurring in the human genome, SNPs are the most abundant, occurring approximately one in every 300–1,000 bp along the three billion bp human genome, and the evolutionarily most stable polymorphic variants.

A number of genetic studies have identified important protective roles for various erythrocytic variants and immune response genes that influence susceptibility to malaria (reviewed in Hill 2006). An array of common variants that confer resistance to malaria infection have been identified, including Hb variants (e.g., Hb S, Hb C, Hb E), α- and β-thalassemias, glucose-6-phosphate dehydrogenase (G6PD) deficiency, and the null allele of the Duffy blood group (Kwiatkowski 2005). The majority of these variants possess a strong genetic signature of selection (Hamblin et al. 2002; Ohashi et al. 2004; Saunders et al. 2005; Wood et al. 2005) and reviewed in (Hancock and Rienzo 2008).

Over the past three decades, it has become apparent that variability in host immune response genes conditions susceptibility to malaria disease outcomes. As such, there has been an accelerated effort to investigate variation in key immune response pathways that mediate innate and acquired immunity. Numerous studies illustrate the importance of cytokines, chemokines, and effector molecules with severe malaria outcomes (see Table 8.1).

12.1 Candidate Gene Approach

The candidate gene approach, which employs the targeted investigation of a particular gene/gene pathway, is a viable approach for gaining an improved understanding of the molecular mechanisms that condition susceptibility to the acquisition of disease, as well as the differing clinical outcomes that result from an infection. We have successfully utilized this approach to more fully understand how innate immune response genes govern the clinical outcomes in children with *P. falciparum* malaria once they become infected. For example, since nearly 100% of the infants and children in holoendemic *P. falciparum* transmission regions have repeated malaria infections, the question is not "what protects against acquisition of malaria," but rather "what genes/gene pathways are associated with susceptibility to SMA." Such an approach offers some unique statistical advantages over more broad-based technologies that investigate millions of different variants, and thereby, require more sophisticated bioinformatic and statistical tools. Through the candidate gene approach, focused specifically on innate immune response genes in children with malaria, we have identified a number of novel and known genes that condition susceptibility to SMA, and also mediate functional changes in their respective gene products. The results of these studies, as well as a large number of others can be found in Table 8.1. In addition, the following sections listed below provide a focused discussion of a selected number of these polymorphic variants as illustrative examples. The choice of inclusion was driven by the desire to link the genetic findings with the comprehensive discussion of innate and acquired immunity discussed above.

In the context of innate immunity, we recently identified a novel functional variant in the SCGF promoter (−539 C/T) that protects children against SMA (Ouma et al. 2010). Additionally, we have also shown that variation in the promoter region of MIF (−173 G/C) is associated with an increased risk of high-density parasitemia (HDP ≥ 10,000 mps/μL) (Awandare et al. 2006c). In the context of acquired immunity, our laboratories demonstrated a protective effect of the FcγRIIa-H131 genotype against development of HDP once children become infected with falciparum malaria (Ouma et al. 2006). These results are similar to another study performed in a neighboring region of Kenya in which polymorphic variability in FcγRIIa protected infants against HDP (Shi et al. 2001). In contrast, an investigation in West Africans showed that the FcγRIIa HH131 genotype increased susceptibility to severe malaria (Cooke et al. 2003). Another study in Thai patients with falciparum malaria also showed that carriage of the FcγRIIa HH131 genotype increased susceptibility to CM (Omi et al. 2002). These results underscore a common theme when investigating the genetic basis of susceptibility to malaria disease outcomes: results are highly variable across different ethnic groups and in differing regions of malaria endemicity.

Table 8.1 Association between genetic variation and malaria

Gene	SNP	Population	Disease association	Outcome	Reference
CD40L	−726 C>T	Gambian individuals ($n=957$)	Severe malaria	Protection against severe malaria	Sabeti et al. (2002)
Cytokine-inducible SRC homology 2 (SH2) domain protein (CISH)	−639 −292 A>T −163 +3414	Gambian, Hong Kong, Kenyan, Malawian and Vietnamese ($n=8,402$)	Severe malaria	Susceptibility to severe malaria	Khor et al. (2010)
FcγRIIa (CD32)	−131 H>R	Gambian children ($n=1,415$; 333-mild malaria, 524 severe malaria and 558 non-malaria controls)	Mild malaria/severe malaria	HH131 genotype associated with susceptibility to severe malaria, R131 allele protected against disease (parasitemia)	Cooke et al. (2003)
	131 R>H	Ethnically diverse Indian population ($n=1871$)	Severe malaria in endemic and non-endemic regions	Increased risk of severity of *P. falciparum* malaria	Sinha et al. (2008)
	−131 H>R	Infants from the Asembo bay cohort in Western Kenya ($n=182$; 97 high risk case, 85 low risk control group)	High-density parasitemia (>5,000 mps/μL)	Protection against high density *P. falciparum* infection	Shi et al. (2001)
	−131 H>R	Thai patients ($n=466$; 107-CM, 157-SA and 202 mild malaria controls)	Cerebral malaria/severe malaria	Susceptibility to CM	Omi et al. (2002)
FcγRIIIB	−NA1>NA2				
Heme oxygenase-1	HO-1$(GT)_n$	Thai patients ($n=486$)—329 non-severe malaria, 80 *P. vivax*, 77 severe or CM	Severe malaria/cerebral malaria	No association with malaria pathogenicity, susceptibility and severity	Kuesap et al. (2010)

IFNA2	−305_−300delAACTTT	Five populations (Vietnamese-586; Central Africans-199; Brazilians-265; Kaingang-108 and Guarani-98)	Cerebral malaria	No association	Tena-Tomas et al. (2008)
IL-1	+4845 G>T +3953 C>T	Gambian case control study ($n=1420$; 528 severe malaria, 338 mild malaria and 554 controls)	Mild malaria/severe malaria	Susceptibility to severe malaria	Walley et al. (2004)
IL-1B	−31 C>T 3953 C>T IL1RA VNTR	Thai patients ($n=312$)—(109 CM, 203 mild malaria)	Cerebral malaria/mild malaria	No association	Ohashi et al. (2005)
IL-3 (5q31–33)	+79 C>T	Ghanaian children with *P. falciparum* malaria ($n=1015$)	Recurrent *P. falciparum* malaria	IL-3 79 T allele is associated with risk of recurrent malaria attacks	Meyer et al. (2011)
	−16 T>C	Thai adults ($n=361$)	Severe malaria	No association with severe malaria	Ohashi et al. (2003)
IL-4	−590 T>C	Thai adults ($n=361$)	Severe malaria	No association	Ohashi et al. (2003)
	−590 C>T	Thai-Cambodian ($n=279$; 110-CM and 169 UCM).	CM/uncomplicated malaria	Differential regulation of anti-malarial antibody isotypes (IgG subclasses and IgE)	Tangteerawatana et al. (2009)
	−590 C>T	Fulani and Gogon ethnic tribes of Mali	Malaria infection prevalence	Association with *P. falciparum* prevalence	Vafa et al. (2007)
	VNTR, −33 C>T	Malian children ($n=383$; 240-CM, 101-SM and 42-UM)	Cerebral malaria/ severe malaria/ uncomplicated malaria	Risk factor for SM	Cabantous et al. (2009)

(continued)

Table 8.1 (continued)

Gene	SNP	Population	Disease association	Outcome	Reference
IL-10	−1087 A>G	Fulani and Gogon ethnic tribes of Mali	Malaria infection prevalence	No association	Vafa et al. (2007)
	Individual SNPs (IL-10 +5876 G>A, +4949 A>G, +4251 T>C, +1547 G>A, +919 C>A, −627 T>G, −1117 T>C, −3585 A>T	Gambian children ($n=654$ cases), and 579 sets of parents and 459 matched controls	Severe malaria	No association	Wilson et al. (2005)
	Haplotypes (IL-10 +4949 G/+919 C/−627 G/−1117 C/−3585 T			Protection against SM	
IL-12 and receptors	55 SNPs covering IL12A and IL12B and receptors IL12RB1 and IL12RB2	Children in western Kenya ($n=913$)	SMA	Protein from SMA	Zhang et al. (2010)
IL12B	Several SNPs covering chromosome 5q31–q33, including IL12Bpro and IL12B 3′UTR	Familial study in Burkina Faso ($n=215$ belonging to 34 families)	Parasitemia levels	No association with parasitemia levels	Barbier et al. (2008a)
IL-12p40 (IL12B)	IL12B 3′UTR; IL12Bpro	Thai adults ($n=355$)	SM	Protection against SM	Phawong et al. (2010)

	IL12B 3′UTR; IL12Bpro	Tanzanian children ($n=178$; 86-CM, 55 uncomplicated clinical malaria; 50-asymtomatic malaria exposed HC)/Kenyan children ($n=693$ cases and $n=693$ controls). Of 693 cases, 413-CM, 280-severe anemia, respiratory distress and/or hypoglycemia	Cerebral malaria/severe malaria	Mortality from CM and reduced nitric oxide production among Tanzanian children No association with SM in Kenyan children	Morahan et al. (2002)
IL-13	−1055 C>T	Thai adults ($n=361$)	Severe malaria	Protection against severe malaria	Ohashi et al. (2003)
	82 SNPs spanning 522 kb of the 5q31 region	Thai malaria patients ($n=368$); 203 mild malaria and 165 severe malaria	Mild malaria/severe malaria	No association with severe malaria	Naka et al. (2009)
	+3545 A>C			Associated with severe malaria outcomes	
IL-22	+708 A>G		Severe malaria	Protection against severe malaria	Koch et al. (2005)
	−1394 C>T		Cerebral malaria	Increased susceptibility to CM	
	+708 T/−1394 G haplotype		Severe malaria	Increased susceptibility to e malaria	

(continued)

Table 8.1 (continued)

Gene	SNP	Population	Disease association	Outcome	Reference
NOS2 (iNOS)	−954 G>C	Ugandan children ($n=307$)	Symptomatic malaria	Protection against malaria	Parikh et al. (2004)
	CCTTT microsatellite repeat (>15 repeats)	Thai adults ($n=435$); 256 severe *P. falciparum* malaria and 179 mild malaria	Severe malaria/mild malaria	longer repeats associated with severe malaria	Ohashi et al. (2002)
	−954 G>C	Gabonese children ($n=841$)	Severe malaria (hyperparasitemia and/or severe malaria)	Protection against malaria	Kun et al. (2001)
	Several SNPS ($n=34$) flanking 7.3 kb region of NOS2 promoter	Tanzanian children ($n=1318$)	Cerebral malaria/ uncomplicated malarial	No associations with malarial severity	Levesque et al. (2010)
Lymphotoxin alpha allele (LTA)	LTA +25 2A>G LTA +80 C>A	Burkina Faso ($n=199$) belonging to 34 families	Mild malaria and parasitemia	Associated with parasitemia	Barbier et al. (2008b)
	+252 T>C +80 G>T	Large African cohort ($n>10,000$), drawn from Gambia, Kenya, and Malawi	Severe malaria	TNF haplotypes associated with SM in Gambian population. No association with any other SNPs	Clark et al. (2009)
Monocyte chemoattractant protein 1 (MCP-1)	Five SNPs: −518 A>G −348 G>C −2158 C>T −2076 A>T −2072 T>C	Thai patients ($n=481$) with mild malaria ($n=206$), severe ($n=165$), and cerebral ($n=110$)	Severe malaria/cerebral malaria	No association	Dechkum et al. (2006)
MIF	−173 G>C and −794CATT haplotypes	Kenyan children ($n=643$)	SMA	Protection against SMA	Awandare et al. (2009)

TIRAP (Toll-interleukin-1 receptor domain-containing adaptor protein)	S180L	Iranian malaria patients ($n=640$); 320 *P. falciparum* infected cases and 320 non-infected controls	Mild malaria	Associated with development of mild malaria	Zakeri et al. (2011)
TLR-4	D299G, T399I	Iranian malaria patients ($n=640$); 320 *P. falciparum* infected cases and 320 non-infected controls	Mild malaria	No association with mild malaria	Zakeri et al. (2011)
	Asp299Gly Thr399Ile	Ghanaian children ($n=870$)	Severe malaria	Associated with increased risk of severe malaria	Mockenhaupt et al. (2006)
	Asp299Gly Thr399Ile	Indian patients with mild malaria ($n=293$)	*P. falciparum* blood infection levels (parasitemia)	Associated with low parasite density	Basu et al. (2010)
TLR4 and TLR2	34 SNPs in TLR4 and 12 SNPs TLR2	Ghanaian patients ($n=4292$)	Malaria and mortality in people living under high infectious pressure	Do not influence the susceptibility to malaria infection or mortality	May et al. (2010)
TLR-9	−1486 T>C −1237 T>C	Iranian malaria patients ($n=640$); 320 *P. falciparum* infected cases and 320	Severe malaria	No association with mild malaria	Zakeri et al. (2011)
	−1237 T>C −1486 T>C	Ugandan children	Cerebral malaria	Altered cytokine levels in children with parasitemia	Sam-Agudu et al. (2010)
	(−1486 T>C, −1237 C>T, +1174 A>G and +2848 G>A)	family based study from Malawian and Gambian populations ($n>6000$)	Severe malaria	No association with severe malaria	Campino et al. (2009)

(continued)

Table 8.1 (continued)

Gene	SNP	Population	Disease association	Outcome	Reference
TNF-α	−1031 T>C	Indian patients with mild malaria ($n=293$)	*P. falciparum* blood infection levels (parasitemia)	Associated with low parasite density	Basu et al. (2010)
	−308 G>A	208 Malian families ($n=136$ CM and $n=72$ SM)	Severe malaria/cerebral malaria	No association with severe malaria	Cabantous et al. (2006)
	−238 G>A	384 families ($n=240$ CM and 108 SM)	Severe malaria/cerebral malaria	No association with SM	
	−308 G>A −1031 T>C	Adult Indian patients ($n=52$; 38 cases and 14 HC	*P. vivax* malaria	No increased risk	Sohail et al. (2008)
	−1031 T>C −863 C>A −857 C>T	Thai adult patients ($n=466$)	*P. falciparum* Cerebral Malaria	C (−1031 C, −863 C and −857 C) alleles associated with CM	Hananantachai et al. (2007)
TNF	−1031 T>C −863 C>A −857 C>T −376 G>A −308 G>A −238 G>A	Thai patients ($n=486$)—329 non-severe malaria, 80 *P. vivax*, 77 severe or CM	Severe malaria/cerebral malaria	No association with malaria pathogenicity , susceptibility and severity	Kuesap et al. (2010)
	+851 A>G −238 G>A −308 G>A −376 G>A −1031 T>C	Large African cohort ($n>10,000$), drawn from Gambia, Kenya, and Malawi	Severe malaria	TNF haplotypes associated with SM in Gambian population. No association with any other SNPs	Clark et al. (2009)

TNF-enhancer	Six SNPs −76 T>A −1031 T>C −863 C>A −857 C>T −308 G>A −238 G>A	Ethnically diverse Indian population ($n=1871$)	Severe malaria in endemic and non-endemic regions	Increased risk of severity of *P. falciparum* malaria	Sinha et al. (2008)
TNF/LTA/LTB		Highland Papuan children and adults ($n=380$ cases and 356 controls)	Severe malaria	No associations	Randall et al. (2010)

12.2 Linkage Disequilibrium

Comparative studies of ethnically diverse human populations, particularly in Africa, are imperative for understanding the genetic basis of phenotypic adaptations and complex disease processes (Campbell and Tishkoff 2008). Genetic mapping of complex disease traits relies on the identification of an association between polymorphic markers, either individually or as haplotypes, and disease phenotype (Tishkoff and Verrelli 2003a). LD, the nonrandom association between alleles at different loci, is typically measured using two different estimators: D' and r^2 (Pritchard and Przeworski 2001). Levels and patterns of LD depend on a number of demographic factors including population size and structure, as well as locus-specific factors such as selection, mutation, recombination, and gene conversion (Tishkoff and Verrelli 2003a,b; Pritchard and Przeworski 2001; Abecasis et al. 2005). LD is particularly useful for inferring evolutionary and demographic processes, as well as for mapping disease susceptibility loci. Therefore, an understanding of levels and patterns of LD has broader implications for studies of human evolutionary history and disease (Campbell and Tishkoff 2008).

LD studies have proved important for identifying genetic loci associated with malaria susceptibility, especially in Africa where there is high genetic diversity within populations (Campbell and Tishkoff 2008; Pritchard and Przeworski 2001; Abecasis et al. 2005; Tishkoff and Verrelli 2003b; Conrad et al. 2006; Mackinnon et al. 2005). Since the application of LD associations in genetic-based study designs supplements and compliments the candidate gene approach in case–control studies, we have investigated the association between haplotypes (a combination of two or more alleles at adjacent, but distinct loci on the same chromosome that are inherited together) and susceptibility to severe malaria for a large number of immune response genes. Using this approach, we observed that haplotypes of IL-10 promoter polymorphisms (IL-10 −1082 A/G, −819 T/C and −592 A/C) were associated with susceptibility to SMA and functional changes in circulating inflammatory mediator levels (e.g., IL-10, TNF-α, IL-6 and IL-12) (Ouma et al. 2008b). This observation parallels results in a Gambian population showing a strong protective association between IL-10 haplotypes (+4949 G; +919 C; −627 G; −1117 C; and −3585 T) and severe malaria, CM, and SMA (Wilson et al. 2005). Additionally, we have also demonstrated that haplotypes of MIF promoter polymorphisms (−173 G/C and −794 CATT) are associated with susceptibility to SMA and reduced plasma MIF concentrations (Awandare et al. 2009). These results are consistent with findings in Zambian children showing that carriers of 15 CATT repeat alleles at −794 are at a greater risk of developing HDP relative to individuals with only five repeats (Zhong et al. 2005).

Similarly, haplotypic constructs of IL-1β promoter variants (−31 C/T and −511 A/G) that condition lower levels of IL-1β production are associated with increased risk of developing SMA (Ouma et al. 2008a). These results are not universal since other studies failed to demonstrate any significant relationships between haplotypes of IL-1β (and IL-1β receptor polymorphisms) and either SMA or CM in Ghanaian and Gambian children, and Thai adults (Cooke et al. 2003; Ohashi et al. 2005; Gyan et al. 2002).

One of our recent investigations in Thai adults showed that haplotypes of *IL12B* polymorphisms (i.e., *IL12B*pro and *IL12B* 3′UTR) conditioned susceptibility to severe malaria and functional changes in circulating IL-12p40 and IFN-γ levels (Phawong et al. 2010). These results are similar to a previous study among Tanzanian children in which *IL-12B* promoter polymorphisms were associated with elevated levels of NO and CM-associated mortality (Morahan et al. 2002). A more recent family association study further demonstrated that the functional promoter variants of *IL-12Bpro* conditioned susceptibility to CM (Marquet et al. 2008). In addition, a comprehensive longitudinal study in western Kenya utilized 55 tagging SNPs (covering genes encoding *IL12A*, *IL12B*, *IL12RB1*, and *IL12RB2*) to demonstrate a number of important associations between genetic variation and SMA (Zhang et al. 2010). Our recent study in western Kenya also showed that variation in *IL12B* 3′UTR was associated with susceptibility to SMA, and although IL-12p40 was reduced in children with severe anemia, *IL12B* 3′UTR was not associated with functional differences in IL-12p40 production when stratified according to genotypes (Ong'echa et al. 2011a). In contrast to the above studies demonstrating a significant association between IL-12 variants and malaria disease susceptibility, a recent investigation focusing on family lineage in Burkina Faso found no relationship between *IL-12B* variants and *P. falciparum* parasite density (Barbier et al. 2008b).

With the advent of improved and more robust SNP genotyping platforms, genetic associations based on LD application has enabled a broader analysis of genetic linkages. Analyses of the TNF/lymphotoxin-α/lymphotoxin-β (i.e., TNF/LTA/LTB) locus found no significant relationships between variability and severe malaria in the highlands of Papua New Guinea in either children or adults (Randall et al. 2010). These results are comparable with results from a GWA study that involved three African populations from The Gambia, Kenya, and Malawi (Clark et al. 2009). Although there were no significant associations between the TNF/LTA variants and severe malaria in the Kenyan and Malawian populations, the TNF −238 polymorphism was associated with an increased the risk of developing severe malaria in the Gambian population (Clark et al. 2009). However, neither the −308 nor the −238 alleles were associated with severe malaria in Malian children (Cabantous et al. 2006). Additional work has shown that the A allele at TNF −376 increases susceptibility to CM in both Gambian and Kenyan populations (Knight et al. 1999). Taken together, these results illustrate that polymorphisms within the TNF gene locus differentially regulate malaria pathogenesis.

12.3 GWA Studies

A GWA study (or whole genome association study) is a technique that employs a comprehensive cataloging of variation within individuals in all (or most) genes that then links this variation to a particular disease phenotype. This methodology allows for a comparison of how inter-individual variability is associated with particular

disease traits. Over the past 5 years, GWA studies have presented a new powerful approach in discerning the genetic basis of a number of diseases (Donnelly 2008; Manolio et al. 2008; McCarthy et al. 2008). This effort, however, has been met by necessity of developing complex data analytic strategies, particularly in Africa populations with greater amounts of genetic diversity (Conrad et al. 2006; Jakobsson et al. 2008; Tishkoff et al. 2009). Although Africa is considered the ancestral origin of mankind (Campbell and Tishkoff 2008; Tishkoff and Williams 2002), and the geographic region that harbors the vast majority of infectious diseases (Black et al. 2003; Mathers et al. 2009; Mayosi et al. 2009), whole-genome approaches have been challenging in these populations. Interesting results from GWA studies in African populations are beginning to emerge in ethnic populations from Nigeria, Kenya, The Gambia, and Malawi (Jallow et al. 2009; Timmann et al. 2007; Frazer et al. 2007; International-HapMap-Consortium 2005; Joubert et al. 2010).

A comprehensive analyses of genome-wide variation and population substructure was recently reported (Joubert et al. 2010). Although this study demonstrated no strong genetic variability associated with self-reported ethnicity, there was clearly a distinct genetic difference among populations of African ancestry reported in the International HapMap project, supporting previous documentation of genetic diversity among African populations (Campbell and Tishkoff 2008; Conrad et al. 2006; Jakobsson et al. 2008; Tishkoff et al. 2009; Tishkoff and Williams 2002; Jallow et al. 2009).

Although numerous studies have identified regions of the genome that affect resistance, or conversely, susceptibility to a variety of common diseases, one of the first GWA studies on malaria in African populations was performed in individuals with mild forms of disease (Timmann et al. 2007). This investigation identified genomic regions that were linked to parasitological and clinical phenotypic outcomes (Timmann et al. 2007). An additional GWA study in severe malaria was recently reported in The Gambia, West Africa in which the authors concluded that genetic variability among African populations, coupled with the presence of weak LD between genetic variants and phenotypic outcomes required distinct and more robust methodological strategies for GWA studies in African populations than those commonly applied in European and Asian populations (Jallow et al. 2009). Results from this investigation provide "proof of principle" that causal variants can be identified by multipoint imputation based on population-specific deep sequencing data (Jallow et al. 2009; Teo et al. 2010).

In summary, a number of genetic markers have been identified that condition susceptibility to malaria, but are, however, highly variable across populations and within differing areas of malaria endemicity. These findings underscore the fact that development of a malaria vaccine that provides protection for diverse ethnic populations residing in differing geographic regions will prove very challenging. However, successful and comprehensive cataloging of the genetic variants that most strongly influence malaria disease outcome can be maximized by having a phenotypically well-defined/stratified population.

13 Modeling of Human Malaria Using Genetic- and Immunological-Based Data

As part of our overall objective of determining the clinical, genetic, and immunological predictors of clinical outcomes in children with malaria, we have utilized a variety of statistical techniques, the specifics of which depend on the particular question and the temporal structure of the data (i.e., cross-sectional versus longitudinal). As regards to cross-sectional data analysis, many of our research questions entail comparisons of group means and medians. Our approach to analyzing such comparisons depends on the distributional characteristics of the target variables. When the data are approximately normally distributed and parametric assumptions are met, group mean differences are examined via analysis of variance (ANOVA) or analysis of covariance (ANCOVA), depending on whether covariates or control variables are modeled. When the ANOVA/ANCOVA omnibus *F*-test is significant, post-hoc pairwise comparisons, with familywise alpha-rate adjustment, if needed, are performed to examine specific subgroup mean contrasts. Alternatively, if focused pairwise mean comparisons are of a priori interest then we perform multiple parametric *t*-tests, again with familywise alpha rate adjustment, if needed. When variables are non-normally distributed and hence do *not* meet parametric assumptions, we opt for one of two approaches. First, depending on the extent of non-normality, we may transform our variables toward normality (using, for example, a logarithmic transformation) and then proceed to analyze the data using parametric statistical techniques. Second, we might, and often do, analyze the non-normal variables using nonparametric statistical methods. In this case, group means are examined using the Kruskal–Wallis ANOVA technique, and pairwise mean contrasts are performed via Mann–Whitney *U*-tests. In addition to conducting group mean comparisons, we are also interested, at times, in examining group differences in proportional measurements. In these situations, we perform either Pearson's chi-square test or Fisher's exact test, depending on the group sample sizes.

Additional approaches that we use to analyze cross-sectional data are geared toward model testing, with a particular emphasis on uncovering important predictive associations. In this context, we largely rely on correlation- and regression-based modeling. In particular, Pearson and Spearman correlation coefficients are used to examine bivariate associations, and regression analysis is performed to simultaneously examine multiple predictor variables. Much of our regression-based modeling entails fitting and evaluating one or more linear hierarchical multiple regression models. Such models are "hierarchical" in the sense that control variables are entered into the model first (i.e., their criterion variance is accounted for), before examining the contribution of the predictor variables of interest. In addition to quantifying the percent of criterion variance accounted for by the predictors, we also examine the predictive strength of the individual component predictors. Two specific indices that we evaluate in this regard are standardized partial regression coefficients and squared semipartial correlation coefficients. Briefly defined, a standardized partial regression coefficient, also known as a beta-weight, represents the

influence of a single predictor on an outcome, controlling for all other predictors. Formally, a beta-weight indicates how many standard deviations change are expected in the outcome variable when there is a one standard deviation change in the predictor variable (controlling for all other predictors). The squared semipartial correlation represents the unique amount of criterion, or outcome, variance accounted for by a given predictor variable (again, controlling for all other predictors). Linear hierarchical multiple regression is appropriate for use with continuously-scaled outcome variables. When outcome measures are categorical, other regression techniques, such as logistic regression, must be used. Because our research team is interested in characterizing distinct clinical phenotypes (e.g., children with severe versus mild malarial anemia) as well as groups with different genetic profiles (e.g., distinct genotypes or haplotypes), we often model categorical (phenotypic) outcomes using logistic regression analysis. Similar to our linear regression modeling approach, we often examine the results of hierarchical logistic regression models. In these analyses, we evaluate the statistical significance of a predictor by inspecting the Wald coefficient and accompanying *p*-value, and we estimate the direction and magnitude of predictive effects by examining odds ratios along with corresponding 95% confidence intervals.

Our approach to analyzing longitudinal outcomes, such as mortality rates and repeated episodes of SMA, also relies heavily on regression-based modeling. Similar to the strategy outlined above, we use hierarchical logistic regression to examine binomial/dichotomous and multinomial outcomes. From such analyses, we often interpret both odds ratios and relative risk ratios. In addition to logistic regression, we perform other regression modeling procedures, most notably Poisson regression and Cox regression. Poisson regression is appropriate for analyzing counts and rates, and it, along with negative binomial regression, is the preferred approach for analyzing zero-inflated data (i.e., data that are characterized by a preponderance of non-events or "zeros," and very few actual events). Mortality rate over time in an epidemiological cohort such as ours is a classic example of a zero-inflated variable, although some children die over time, the vast majority will survive. Cox regression (survival analysis) is also an important tool for analyzing longitudinal outcomes. In our studies, we use Cox regression to estimate group-specific hazard rates (i.e., the probabilities of experiencing the event over time, such as death), and then compare the mean, or median, hazard rates across groups using a nonparametric test such as the Kolmogorov–Smirnov test.

Finally, an important data-analytic challenge that we have encountered, and one that will likely increase in frequency with the assimilation of additional high-throughput genomic data and our participation in GWA studies, is the issue of high-dimensional data (i.e., an extremely large predictor, *p*-to-subject, *n* ratio). One strategy in this situation is to perform classic dimension-reduction/data-reduction techniques, such as principal component analysis or cluster analysis, and then analyze the resulting components or clusters using traditional parametric and non-parametric statistical techniques. An example of such analyses can be found in our recent publication (Ong'echa et al. 2011b). A second option, but one that has been used far less frequently in the literature, is to analyze predictive associations using

robust statistical methods that are appropriate for high-dimensional data analysis. Two such procedures that we have employed are least angle regression (LAR) (Efron et al. 2004) and shotgun stochastic search (SSS) (Hans et al. 2007). LAR implements a regression algorithm for high-dimensional data that utilizes a variant of forward stepwise regression to select a parsimonious set of predictors, from a large number of possible predictors, for efficient prediction of a response variable. SSS, on the other hand, implements an algorithmic approach that is similar to Markov Chain Monte Carlo (MCMC), but is generally more effective than MCMC at exploring the vast space of possible regression models and at aggressively narrowing in on the best sets of candidate predictor variables. Simulation studies indicate that both LAR and SSS are efficient and accurate procedures for identifying the best sets of predictor variables. An example of the use of LARS in biological datasets can be found in our recent publication (Ong'echa et al. 2011b).

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